

DEPARTMENT OF FOOD & INDUSTRIAL MICROBIOLOGY

STUDY MATERIAL

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LECTURE-1

Microbial spoilage of foods. Cause of spoilage classification of foods by ease of spoilage.

Factors affecting kinds and numbers of microorganisms in food.

Spoilage of food by micro organisms

Cause of spoilage:

Spoilage may be due to one or more of the following

1. Growth and activity of micro organisms
2. Insects
3. Action of the enzymes of the plant or animal food.
4. Purely chemical reactions i.e., those not catalysed by enzymes of the tissues or of micro organisms
5. Physical changes such as those caused by freezing, burning, drying, pressure etc.

Classification of foods by ease of spoilage:

1. Stable or non perishable foods:

These foods which do not spoil unless handled carelessly include such products as sugar, flour and dry beans.

2. Semi perishable foods:

If these foods are properly handled and stored, they will remain unspoiled for a fairly long period.

Ex: Potatoes, apples etc.

3. Perishable foods:

It includes most important daily foods that spoil readily

Ex: Meats, fish, poultry, milk, eggs, fruits and vegetables.

Factors affecting kinds and numbers of micro organisms in food:

Kind of spoilage of foods by micro organisms and enzymes will depend on the kinds and numbers of these agents present and on the environment about them.

Most raw foods contain a variety of bacteria, yeasts and molds and may contain plant and animal enzymes as the case may be.

Kind and number of micro organisms that will be present on or in food will be influenced by the kind and extent of contamination.

Because of the environmental conditions, one organisms it spoilage by the first organism allowed to proceed, one or more other kinds of organisms produces secondary spoilage or a further succession of organisms and changes may be involved.

Contamination may increase number of micro organisms in the food. Wash water may add surface bacteria in butter, plant equipment may add spoilage organisms to foods during processing. Increased “bio burden” of micro organisms, especially those which cause spoilage, makes preservation more difficult.

Growth of micro organisms in food obviously will increase number of micro organisms.

Pretreatment of foods may remove or destroy some kinds of micro organisms and inactivate part or all of the food enzymes.

Washing may remove organisms from the surface or may add some from the wash water. If washing is by means of an antiseptic or germicidal solution, numbers of organisms may be greatly reduced. High temperatures will kill more organisms treatment with rays, ozone, SO₂, germicidal vapors will reduce numbers.

Factors affecting the growth of micro organisms in food:

Associative growth:

Associations of micro organisms with each other are involved in spoilage or fermentation of most foods.

Competition between the different kinds of bacteria, yeasts and molds in a food ordinarily determines which one will outgrow the others and cause its characteristic type of spoilage.

If conditions are favourable for all, bacteria usually grow faster than yeasts and yeasts faster than molds.

Micro organisms are not always **antagonistic** or antibiotic to each other, however and may sometimes be symbiotic i.e., mutually helpful.

Some times they are **synergistic** i.e., when growing together they may be able to bring about changes such as fermentations.

Ex: *Pseudomonas syncyanea* growing alone in milk produce light brownish tinge and *streptococcus lactis* no color change in milk. When two organisms grow together, a bright blue color develops resulting from P^H effect on the brown pigment produced by *P. syncyanea*.

Metabiosis in which when one organism makes conditions favourable for growth of the second. Both organisms may be growing at the same time, but more commonly one succeed the other.

Ex: Raw milk at room temperature normally first supports an acid fermentation by *streptococcus lactis* and *coli form bacteria* until the bacteria are inhibited by the acid they have produced. Next the acid folerant *lactobacilli* increase the acidity further until they are stopped. Then film yeasts

and molds grow over the top, finally reducing the acidity so that *proteolytic bacteria* can become active.

Effect of environmental conditions:

Environment determines which the different kinds of micro organisms in a food will outgrow the others and causes its characteristic type of change or spoilage. Chief among these factors are the intrinsic or chemical properties and extrinsic or physical properties of the food.

LECTURE-2

Factors affecting growth and survival of microorganisms in foods. Intrinsic factors – Nutrient content, pH, buffering capacity, Redox potential (Eh), Inhibitory substances and biological structures (Antimicrobial barriers and constituents) water activity.

Factors affecting the growth and survival of micro-organisms in foods.

Intrinsic parameters:

The parameters of plant and animal tissues that are inherent part of the tissues are referred to as intrinsic parameter. These parameters are as follows:

1. pH:

PH: It is the negative logarithm of the hydrogen ion activity.

$$\begin{aligned} \text{PH} &= -\log (a_H) = \log \frac{1}{(a_H)} \\ &= \log \left[H^{-1} \right] \end{aligned}$$

pH = Hydrogen ion activity

$[H^+]$ = Hydrogen ion concentration.

Every micro organism has a minimal, a maximal and an optimal pH for growth.

Bacteria grow fastest in the pH range 6.0 – 8.0, yeasts 4.5 – 6.0 and filamentous fungi 3.5 – 4.0.

Usually between pH 5.0 & 6.0.

Inherent acidity:

Some foods have a low pH because of inherent property of the food. Ex: Fruits & vegetables.

Biological acidity:

Some foods develop acidity from the accumulation of acid during fermentation. Ex: curd, sauerkraut, pickles etc.

Molds can grow over a wide range of pH values than the yeast and bacteria. Film yeasts grow well on acid foods such as sauerkraut and pickles. Most yeasts do not grow well in alkaline substrates.

Bacteria which are acid formers are favoured by moderate acidity. Active proteolytic bacteria, can grow in media with a high pH (alkaline.) Ex: Egg white.

The compounds that resist changes in pH are important not only for their buffering capacity but also for their ability to be especially effective within a certain pH range.

Vegetable juices have low buffering power, permitting an appreciable decrease in pH with the production of small amount of acid by lactic acid bacteria during the early part of sauerkraut and pickle fermentations. This enables the lactics to suppress the undesirable pectin – hydrolyzing and proteolytic competing organisms. Low buffering power makes for a more rapidly appearing succession of micro-organisms during fermentation than high buffering power. Ex: Milk – High in protein content, act as good buffer. Lactic acid converted to pyruvic acid by glycolytic pathway. Acid again converts to lactic acid by lactic dehydrogenase enzyme. After 5-10 minutes, there will be decreased in pH. Hence the lactic acid bacteria survives and activity slows down. Once the acidity increase, yeasts and molds will take upper hand and all the products used by these organisms. The quantity of acid decreases and pH increases to neutral. Proteolytic bacteria acts on caesin and these proteins are broken down and gives bad smell accompanied by removal of NH_3 . pH increases and neutral due to deamination. Then lipolytic organisms which utilise the fat present and utilises the short chain fatty acids through hydrolysis which gives still bad smell.

Egg white where the pH increases to around 9.2 as CO_2 is lost from the egg after laying.

Fish spoil more rapidly than meat under chill conditions. The pH of post – rigor mammalian muscle, round 5.6 and it is lower than that of fish (6.2 - 6.5) and this contributes to the longer storage life of meat.

The ability of low pH to restrict microbial growth has been employed since the earliest times in the presentation of foods with acetic and lactic acids.

Fruits are acidic than vegetables pH of milk – neutral.

Fruits generally undergo mold and yeast spoilage than vegetables.

Redox potential (E_h): - Oxidation – reduction potential:

Oxygen tension or partial pressure of oxygen about a food and the O R potential or reducing and oxidising power of the food itself, influence the type of organisms which will grow and hence the changes produced in the food. The O R potential of the food is determined by

1. Characteristic O R potential of the original food.
2. The poisoning capacity i.e., the resistance to change in potential of the food.
3. The oxygen tension of the atmosphere about the food.
4. The access which the atmosphere has to the food.

Head space in an “evacuated” can of food contain low oxygen tension compared to air.

Micro organisms are classified as aerobic, anaerobic, and facultative based on the requirement of O_2 . Molds – aerobic

Yeasts – Aerobic and facultative.

Bacteria – Aerobic, anaerobic and facultative.

High O - R potential favours aerobes and facultative organisms.

Low O-R potential favours anaerobic and facultative organisms.

However some aerobes grow at low O-R potential O-R potential of a system is usually written Eh and measured and expressed in terms of millivolts (mv).

Highly oxidised substrate would have a positive Eh and a reduced substrate have a negative Eh.

Aerobic microorganisms require positive Eh. Ex: Bacillus, micrococcus, pseudomonads acinetobacters.

Anaerobic micro organisms required negative Eh. Ex: Clostridium

Most fresh plant and animal foods have a low and well poised O – R potential in their interior because plants contain reducing substances like ascorbic acid and reducing sugars where as animal tissues contain –SH (Sulf hydryl) and other reducing groups. As long as the plant or animal cells respire and remain active, they have low level of O-R potential.

Meat could support the aerobic growth of shine forming or souring bacteria at the same time that anaerobic putrefaction was proceeding in the interior.

Heating and processing may alter the reducing and oxidising substances of food.

Ex: Fruit juices lost reducing substances by their removal during extraction and filtration by their removal during extraction and filtration and therefore have become more favourable for the growth of yeasts.

3. Nutrient content:

Food is required for energy and growth of micro organisms.

Carbohydrates especially the sugars are commonly used as an energy source. Complex carbohydrates such as cellulose can be utilized by few organisms and starch can be hydrolysed by any a limited number of organisms. Many organisms cannot use the disaccharide lactose (Milk sugar) and therefore do not grow well in milk.

Maltose is not attacked by some yeasts. Some micro organisms hydrolyze pectin of the fruits and vegetables.

Limited number of micro organisms can obtain their energy from fats by producing lipases. Aerobic their energy from fats by producing lipases. Fats are hydrolysed to glycerol and fatty acids. Aerobic micro organisms are more commonly involved in the decomposition of fats than are anaerobic ones and the lipolytic organisms usually are also proteolytic.

Hydrolysis products of proteins, peptides and amino acids serve as an energy source for many proteolytic organisms when a better energy source is lacking. Meats are decomposed by proteolytic sps Ex: *Pseudomonas* sps:

Concentration of food in solution increases the osmotic effect and amount of available moisture. Molds & yeasts can grow in the highest concentrations of sugars. Bacteria can grow best in low concentration of sugars.

Micro organisms differ in their ability to use various nitrogenous compounds as a source of nitrogen for growth. Many organisms are unable to hydrolyze proteins and hence cannot get nitrogen from them. Peptides, aminoacids, urea, ammonia and other simpler nitrogenous compounds may be available to some organisms but not to others. These compounds may be used under some environmental conditions but not under other conditions.

Ex: Some lactic acid bacteria grow best with polypeptides as nitrogen foods, cannot attack casein.

Some microorganisms use fermentable carbohydrates and results in acid production which suppresses the proteolytic bacteria and hence it is called **sparing action** on the nitrogen compounds.

Many kinds of molds are proteolytic but very few yeasts are actively proteolytic. Proteolytic bacteria grow best at pH values near neutrality and are inhibited by acidity. Carbon for growth may come partly from CO₂ and also from organic compounds. Minerals required by microorganisms are always present in low level. Sometimes an essential mineral may be unavailable, lacking or present in insufficient amounts.

Ex: Milk contains insufficient iron for pigmentation of the spores of *Penicillium roqueforti*.

Accessory food substances or vitamins needed by the organisms. Some micro organisms are unable to manufacture some vitamins.

Meats are high in B vitamins and fruits are low, but fruits are high in ascorbic acid.

Egg white contains biotin but also contains avidin. This avidin ties up biotin making it unavailable to microorganisms and eliminating possible spoilage organisms.

Thiamine, pantothenic acid, folic acid group and ascorbic acid are heat labile and drying causes loss in these compounds.

Storage of foods for long periods may result in decrease in level of the accessory growth factors.

Each kind of bacterium has a definite range of food requirements. Some micro organisms can use other carbon compounds such as organic acids and their salts, alcohols and esters.

Pseudomonas spp may be satisfied by simple compounds such as ammonia or nitrates or more complex compounds such as amino acids, peptides or proteins.

Antimicrobial barriers and constituents (or) Inhibitory substances and biological structure:

Inhibitory substances: These originally present in the food or added purposely to prevent growth of micro organisms.

Freshly drawn milk – Lactenins, anticoliform factors.

Egg white – Lysozyme

Cran berries – Benzoic acid

Short chain fatty acids on animal skin cabbage and other brassicas, garlic, onions and leeks.

Allicin – Garlic, onion, leeks.

Phytoalexins are produced by many plants in response to microbial invasion.

Antifungal compound phaseolin produced in green beans

Eugenol – Allspice (pimento), cloves, cinnamon

Thymol – thyme and oregano

Cinnamic aldehyde – cinnamon and Cassia

Inclusion of cinnamon in raisin bread retards mould spoilage.

Humulones contained in the hop resin and isomers produced during processing, impart the characteristic bitterness of beer.

Oleuropein – The bitter principle of green olives have antimicrobial properties.

Lysozyme present in milk, egg is most active against gram positive bacteria.

Egg – Ovotransferrin, avidin ovalaroprotein.

Milk – Lactoferrin

Ovoflavo protein and avidin in egg white which sequester biotin and riboflavin restricting the growth of those bacteria.

Milk has capacity to generate antimicrobials in the presence of hydrogen peroxide. The milk enzyme lactoperoxidase will catalyse the oxidation of thiocyanate by H_2O_2 to produce inter alia Hypo-thiocyanate. This can kill gram negative bacteria and inhibit gram positives.

Propionic acid produced by the propionibacteria in a swiss cheese is inhibitory to molds.

Nisin produced by certain strains of *Streptococcus lactis* may be useful in inhibiting lactate fermenting, gas forming clostridia in curing cheese. Heating foods may result in the formation of inhibitory substances. Ex: Heating lipids may hasten auto oxidation and make them inhibitory.

Browning concentrated sugar syrups may result in the production of furfural and hydroxyl methyl furfural which are inhibitory to fermenting organisms.

Biological structures of food on the protection of foods against spoilage has been observed.

Ex: 1) Inner parts of healthy tissues of living plants and animals are sterile or low in microbial content.

2) Protective covering on the food like shell on egg, skin on poultry, shell on nuts, rind or skin on fruits and vegetables, artificial coating like plastic or wax.

3) Layers of fat over meat may protect the part of the flesh or scales may protect the outer part of the fish.

Water activity:

Micro organisms have an absolute demand for water. Without water, no growth can occur. The exact amount of water needed for growth of micro organisms varies. This water requirement is best expressed in terms of available water or water activity (a_w).

$$a_w = \frac{\text{Vapour pressure of the solution}}{\text{Vapour pressure of the solvent}}$$

a_w for pure water is 1.00

For 1.0 m solution of the ideal solute, the a_w would be 0.9823.

Water activity also defined as the ratio of the partial pressure of water in the atmosphere in equilibrium with the substrate, P , compared with the partial pressure of the atmosphere in equilibrium with pure water at the same temperature, P_0 .

$$A_w = \frac{P}{P_0} = \frac{1}{100} ERH$$

ERH = Equilibrium relative humidity.

A_w Values	
0.98 and above	Fresh meat, fish, fresh fruits and vegetables, milk, canned vegetables, in brine, canned fruits in light syrup.
0.93 – 0.98	Evaporated milk, tomato paste, processed cheese, bread, canned cured meats, fermented sausage, gouda cheese.

0.85 – 0.93	Dried beef, raw ham, aged cheddar cheese, sweetened condensed milk, dry or fermented sausage.
0.60 – 0.85	Dried fruit, flour, cereals, jams & jellies, nuts.
Below 0.60	Chocolate, confectionary, Honey, Biscuits, Crackers, Potato chips, Dried eggs, milk and vegetables.

Water is made unavailable in various ways:

1. Solutes and ions tie up water in solution. Therefore an increase in the concentration of dissolved substances such as sugars and salts effectively dry the material. Water tends to leave the microbial cell by osmosis.
2. Hydrophilic colloids (gels) make water unavailable.
3. Water of crystallization or hydration is usually unavailable to micro organisms.

Each micro organisms has a maximal, optimal and minimal a_w for growth.

Low a_w – decrease in the rate of growth of organisms.

Factors that may affect water activity (a_w). Requirements of micro organisms include the following.

1. The kind of solute employed to reduce a_w . Potassium chloride usually less toxic than NaCl. And less inhibitory than sodium sulphate.
2. The nutritive value of the culture medium. The better the medium for growth, the lower the limiting a_w .
3. Temperature: Most organisms have the greatest tolerance to low a_w at about optimal temperatures.
4. Oxygen supply: Growth of aerobes takes place at lower a_w in the presence of air than in its absence.
5. pH Most organisms are more tolerant of low a_w at pH values near neutrality than in acid or alkaline media.
6. Inhibitors: The presence of inhibitors narrows the range of a_w for growth of micro organisms.

Methods for the control of a_w are

1. Equilibrium with controlling solutions
2. Determination of the water – sorption isotherm for the food.
3. Addition of solutes.

Methods for measuring or establishing a_w values of food:

1. Freezing point determinations by Clausius – Clayperson equation.
2. Manometric techniques
3. Electrical devices.

Favourable a_w for bacteria to grow in foods – 0.995 to 0.998. They grow best in low concentration of sugar or salt. 3-4% sugar and 1-2% salt may inhibit some bacteria.

Molds have optimum a_w of 0.98 – 0.99; Mold spores germinate at min a_w of 0.62.

Some general conclusions related to water requirement of micro organisms are

1. Each organism has its own characteristic optimal a_w .
2. Bacteria require more moisture than yeasts and yeasts more than molds.

Minimum a_w required for bacteria – 0.91

Minimum a_w required for yeasts – 0.88

Minimum a_w required for molds – 0.80

Minimum a_w required for Halophilic bacteria – 0.75

Minimum a_w required for Xerophilic fungi – 0.65

Minimum a_w required for Osmophilic yeasts – 0.60

3. Micro organisms that can grow in high concentrations of solutes e.g. sugar and salt have low water activity (a_w). Osmophilic yeasts grow best in high concentrations of sugar.

LECTURE-3

Extrinsic factors – Relative Humidity, Temperature, Gaseous Atmosphere. Chemical changes caused by microorganisms – changes in nitrogenous organic compounds, non-nitrogenous organic compounds, organic acids, other compounds, Lipids, Pectic substances

Extrinsic parameters (Environmental limitations)

1) Relative humidity: (RH)

Relative humidity and water activity are interrelated. When food commodities having low water activity are stored in an atmosphere of high RH water will transfer from the gas phase to the food. It may take a very long time for the bulk of the commodity to increase in water activity. Once micro organisms have started to grow and become physiologically active they usually produce water as an end product of respiration.

Ex: Grain silos or in tanks in which concentrates and syrups are stored.

Storage of fresh fruits and vegetables requires very careful control of relative humidity. If RH is too low, many vegetables will lose water and become flaccid. If it is too high then condensation may occur and microbial spoilage may be initiated.

2. Temperature:

Microbial growth can occur over a temperature range from about -8°C up to 100°C. at atmospheric pressure.

Thermophiles have optimum - 55-75°C

Mesophile have optimum - 30 -40°C

Psychrophiles (Obligate psychrophiles) – 12 - 15

Psychotroph (facultative) – 25-30

Micro organisms can be classified into several physiological groups based on their cardinal temperatures. Low temperature affects the uptake and supply of nutrients to enzyme systems within the cell. Many microorganisms respond to growth at lower temperature by increasing the amount of unsaturated fatty acids in their membrane lipids and that psychrotrophs generally have higher level of unsaturation in a fatty acid decreases its melting point so that membranes containing higher levels of unsaturated fatty acid will remain fluid and hence functional at lower temperatures.

As the temperature increases above the optimum, the growth rate declines as a result of denaturation of proteins.

Gaseous atmosphere:

Oxygen comprises 21% of the earth's atmosphere and is the most important gas in contact with food under normal circumstances. The inhibitory effect of CO₂ on microbial growth is applied in modified atmosphere packing of food and is an advantage in carbonated mineral waters and soft drinks.

Moulds and bacteria are sensitive to CO₂ condensation. Some yeasts such as *Bettanomyces* spp have tolerance to high CO₂ levels.

Growth inhibition is usually greater under aerobic conditions than anaerobic and the inhibitory effect increases with decrease of temperature, presumably due to the increased solubility of CO₂ at lower temperatures. CO₂ dissolves in water to produce carbonic acid which decreases P^H and partially dissociates into bicarbonate anions and protons. CO₂ also affects solute transport, inhibition of key enzymes involving carboxylation, decarboxylation reactions in which CO₂ is a reactant and reaction with protein amino groups causing change in their properties and activity.

Chemical change caused by micro organisms:

Different chemical changes are possible because great variety of organic compounds are present in foods and numerous kinds of micro organisms that can decompose them may grow in the food.

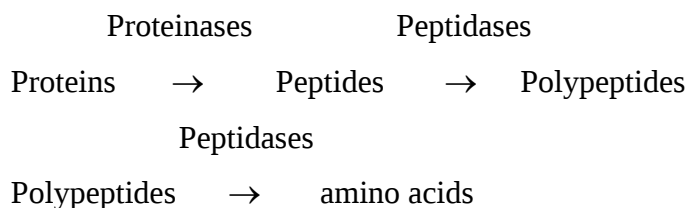
Following changes are observed in foods.

1. Changes in Nitrogenous organic compounds:

Most of the nitrogen in foods is in the form of proteins. Proteins are hydrolysed to polypeptides, simpler peptides or amino acids before they can serve as nitrogenous food for most organisms.

Proteinases catalyze the hydrolysis of proteins to peptides gives bitter taste to foods.

Peptidases catalyze the hydrolysis of polypeptides to simpler peptides and finally to amino acids.



Anaerobic decomposition of proteins, peptides or aminoacids result in the production of obnoxious odors called putrefaction.

Putrefaction results in foul smelling, sulphur containing products such as hydrogen, methyl and ethyl sulfides and mercaptans, plus ammonia, amines (Ex: histamine, tyramine, piperidine, putrescine and cadaverine), indole, skatole and fatty acids.

When micro organisms act on amino acids, they may deaminate them, de-carboxylate them or both.

Ex: *Escherichia coli* produces glyoxylic acid, acetic acid, and ammonia from glycine.

Pseudomonas produces methylamine and CO_2 *clostridia* gives acetic acid, ammonia, methane from alanine these three organisms produces

- 1) α - Keto acid, ammonia and CO_2
- 2) Acetic acid, ammonia and CO_2
- 3) Propionic acid, acetic acid ammonia and CO_2 respectively.

Desulfatocaulum nigrificans an obligate anaerobe, can reduce sulphate to sulphide and produces H_2S from cystine.

Other nitrogenous compounds include

1. Amides, imides and urea from which ammonia is the principal product.
2. Guanidine and creatine which yield urea and ammonia.
3. Amines, purines and pyrimidines which may yield ammonia, CO_2 and organic acids.

Changes in Non nitrogenous organic compounds:

Main non nitrogenous foods for micro organisms, mostly used to obtain energy but possibly serving as source of carbon, include carbohydrates, organic acids, aldehydes and ketones, alcohols, glycosides, cyclic compounds and lipids.

Carbohydrates:

Carbohydrates act as energy source by micro organisms. Complex, di, tri or polysaccharides usually are hydrolyzed to simple sugars before utilization.

A monosaccharide (glucose) aerobically would be oxidised to carbon-dioxide and water.

Glucose anaerobically decompose to

- a) An alcoholic fermentation by yeasts with ethanol and CO_2 as the principal products.
- b) A simple lactic fermentation as by homo-fermentative lactic acid bacteria.
- c) A mixed lactic fermentation by hetero-fermentative lactic acid bacteria with lactic and acetic acids, ethanol, glycerol and CO_2 as the chief products.
- d) The coli type of fermentation as by *coliform bacteria* with lactic, acetic formic acids, ethanol, CO_2 , H_2 etc.
- e) The propionic acid fermentation by propionic bacterium

f) Butyric – butyl – isopropyl fermentations yields butyric and acetic acids, CO₂ & H₂.

Organic acids:

Organic acids usually occurring in foods as salts are oxidized by organisms to carbonates, causing medium to become alkaline. Aerobically the organic acids may be oxidized completely to CO₂ and water. Saturated fatty acids or ketonic derivatives are degraded to acetic acid.

Other compounds:

Alcohols usually oxidised to the corresponding organic acids.

Ethanol to acetic acid; Acetaldehyde to acetic acid.

Lipids:

Fats are hydrolysed to glycerol and fatty acids by lipase.

Phospholipids may be degraded to their constituent phosphate, glycerol, fatty acids and nitrogenous base.

Ex: choline

Pectic substances:

Protopectin in plants converted to pectin.

Pectin is a water soluble polymer of galacturonic acids. Pectinesterase causes hydrolysis of the methyl ester linkage of pectin to yield pectin acid and methanol.

LECTURE-4

Contamination of Foods. Sources of contamination. Green plants and Fruits, Animals, Sewage, Soil, Water, Air

Contamination of foods

Micro organisms from various natural sources act as source of contamination.

From green plants and fruits

From animals

From sewage

From soil

From water

From air

During handling and processing.

1. From green plants and fruits

Natural surface flora of plants varies with the plant but usually includes species of *Pseudomonas*, *Alcaligenes*, *Flavobacterium*, *Micrococcus*, coliforms and lactic acid bacteria. The no. of bacteria will depend on the plant and its environment and may range from a few hundred or thousand per square centimeter of surface to millions.

Ex: Surface of well washed tomato contains 400-700 micro organisms per square centimeter.

Outer tissue of unwashed cabbage contain 1 million to 2 million micro organisms. Inner tissues of cabbage contain fewer micro organisms.

Exposed surface of plants become contaminated from soil, water, sewage, air and animals, so that micro organisms from these sources are added to the natural flora. Whenever conditions for growth of natural flora and contaminants are present, special kinds of micro organisms may increase. Some fruits have been found to contain viable micro organisms in their interior.

2. From animals:

Sources of micro organisms from animals include the surface flora, the flora of the respiratory tract, and the flora of the gastro intestinal tract. Hides, hooves, and hair contain micro organisms from soil, manure, feed and water but contain spoilage organisms.

Feathers, feet of poultry carry heavy contamination of micro organisms.

Skin of many meat animals may contain micrococci, Staphylococci and beta haemolytic streptococci. Pig or beef carcasses may be contaminated with salmonellae.

Salmonellosis associated with eggs has been reduced because of the pasteurization of egg products. Meat from slaughter houses is not frequently associated with human salmonellosis.

Many of these diseases have been reduced or eliminated by improvement in animal husbandry, but animal disease causing infections from foods include *Mycobacterium*, *Coxiella*, *Listeria*, *Salmonella* and enteropathogenic *E.Coli* and viruses.

Insects and birds cause mechanical damage to fruits and vegetables, introduce micro organisms and open the way for microbial spoilage.

3. From sewage:

When untreated domestic sewage is used to fertilize plant crops, there is a chance that raw plant foods will be contaminated with human pathogens especially those causing gastrointestinal diseases. The use of “night soil” as a fertilizer still persists in some parts of the world. In addition to the pathogens, coliform bacteria, anaerobes, enterococci, other intestinal bacteria and viruses can contaminate the foods from this source. Natural water contaminated with sewage contributes their micro organisms to shell fish, fish, and other seafood.

From soil:

Soil contains greatest variety of micro organisms. They are ready to contaminate the surfaces of plants growing on or in them and the surfaces of animals roaming over the land. Soil dust is whipped up by air currents and soil particles are carried by running water to get into or onto foods. Soil is an important source of heat resistant spore forming bacteria.

From water:

Natural water contain not only their natural flora but also microorganisms from soil and possibly from animals or sewage.

Surface waters in streams or pools and stored waters have low microbial content because self purification of quiet lakes and ponds or of running water.

Ground waters from springs or wells have passed through layers of rock and soil to a definite level hence most of the bacteria, suspended material have been removed.

Kinds of bacteria in natural waters are chiefly of in *Pseudomonas*, *Chromobacterium*, *Proteus*, *Micrococcus*, *Bacillus*, *Streptococcus*, *Enterobacter* and *Escherichia coli*.

Two aspects of water bacteriology are Public health aspects and Economic aspects

Public health aspects	Economic aspects
Public health aspects include safe to drink, free from pathogens, water should be tested for coliforms, enterobacter before consumption.	Water is used for processing of fruits & vegetables.

The water commonly is chlorinated but there have been presence of chlorine resistant flora. Efficient filtration greatly reduces the microbial content.

From Air:

Air does not contain a natural flora of micro organisms, but accidentally they are present on suspended solid material or in moisture droplets. Micro organisms get into air on dust or lint, dry soil, spray from stream, lakes or oceans, droplets of moisture from coughing, sneezing or talking and growth of sporulating molds on floors, etc.

Micro organisms in air have no opportunity for growth but merely persist there and the organisms resistant to dessications will live longer. Mold spores because of their small size, resistance to drying and large numbers of per mold plant are usually present in air.

Cocci are more numerous than rod shaped bacteria. Yeasts especially asporogenous chromogenic ones are found in most samples of air.

Number of microorganisms in air at any given time depend on factors like amount of movement, sunshine, humidity, location and the amount of suspended dust or spray.

No. of micro organisms vary from mountains to dusty air. Less on mountains and more in dusty air. Direct rays from the sun kill micro organisms suspended in air and hence reduce numbers. Dry air contains more organisms than moist air. Rain or snow removes organisms from the air.

Number of micro organisms in air may be reduced under natural conditions by sedimentation, sunshine and washing by rain or snow. Filters in ventilating or air conditioning systems prevent the spread of organisms from one part of a plant to another.

During handling and processing:

Additional contamination may come from equipment coming in contact with foods, from packaging materials and from personnel.

LECTURE-5

Microorganisms importance in Food Microbiology. Moulds – General characteristic of moulds, classification and identification of moulds

Microorganisms important in Food microbiology

Molds:

Mold growth on foods, with its fuzzy or cottony appearance, sometimes colored, is familiar to everyone, and usually food with a moldy or "mildewed" food is considered unfit to eat. Special molds are useful in the manufacture of certain foods or ingredients of foods. Thus, some kinds of cheese are mold-ripened, e.g., blue, Roquefort, Camembert, Brie, Gammelost, etc., and molds are used in making Oriental foods, e.g., soy sauce, miso, son-ti, and other discussed later. Molds have been grown as food or feed and are employed to produce products used in foods, such as amylase for bread making or citric acid used in soft drinks. Some molds do produce various toxic metabolites (mycotoxins).

General characteristics of molds:

The term "mold" is a common one applied to certain multicellular filamentous fungi whose growth on foods usually is readily recognized by its fuzzy or cottony appearance. Colored spores are typical of mature mold of some kinds and give color to part or all of the growth. The thallus, or vegetative body, is characteristic of thallophytes, which lack true roots, stems, and leaves.

Morphological Characteristics:

Hyphae and Mycelium The mold thallus consists of a mass of branching, intertwined filaments called hyphae (singular hypha), and the whole mass of these hyphae is known as the mycelium.

The hyphae may be submerged, or growing within the food, or aerial, or growing into the air above the food.

Molds are divided into two groups: septate, i.e., with cross walls dividing the hypha into cells; and noncoenocytic, septate with the hyphae apparently consisting of cylinders without cross walls. The non-septate hyphae have nuclei scattered throughout their length and are considered multicellular.

Special, mycelial structures or parts aid in the identification of molds. Examples are the rhizoids, or "holdfasts," of *Rhizopus* and *Absidia*, the foot cell in *Aspergillus*, and the dichotomous, or Y-shaped, branching in *Geotrichum*.

Reproductive Parts or Structures.

Molds can grow from a transplanted piece of mycelium. Reproduction of molds is chiefly by means of asexual spores. Some molds also *form* sexual spores. Such molds are termed "perfect" and are classified as either *Oomycetes* or *Zygomycetes* if nonseptate, or *Asco-mycetes* or *Basidiomycetes* if septate, in contrast to "imperfect" molds, the Fungi Imperfecti (typically septate), which have only asexual spores.

Asexual Spores

The asexual spores of molds are produced in large numbers and are small, light, and resistant to drying. They are readily spread through the air to alight and start new mold thallus where conditions are favorable. The three principal types of asexual spores are (1) conidia (singular conidium), (2) arthrospores or oidia (singular oidium), and (3) sporangiospores. Conidia are cut off, or bud, from special fertile hyphae called conidiophores and usually are in the open, i.e., not enclosed in any container, in contrast to the sporangiospores, which are in sporangium (plural sporangia), or sac, at the tip of a fertile hypha, the sporangiophore. Arthrospores are formed by fragmentation of a hypha, so that the cells of the hypha become arthrospores. Examples of these three kinds of spores will be given in the discussion of important genera of molds. A fourth kind of asexual spore, the chlamydospore, is formed by many species of molds when a cell here and there in the mycelium stores up reserve food, swell, and forms a thicker wall than that of surrounding cells. This chlamydospore, or resting cell, can withstand unfavourable conditions better than ordinary mold mycelium can and later, under favorable conditions, can grow into a new mold.

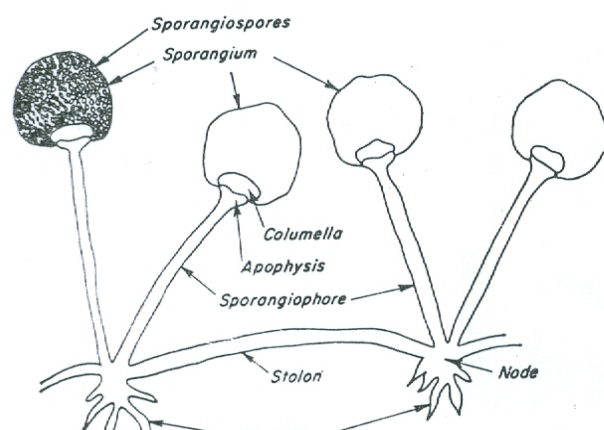
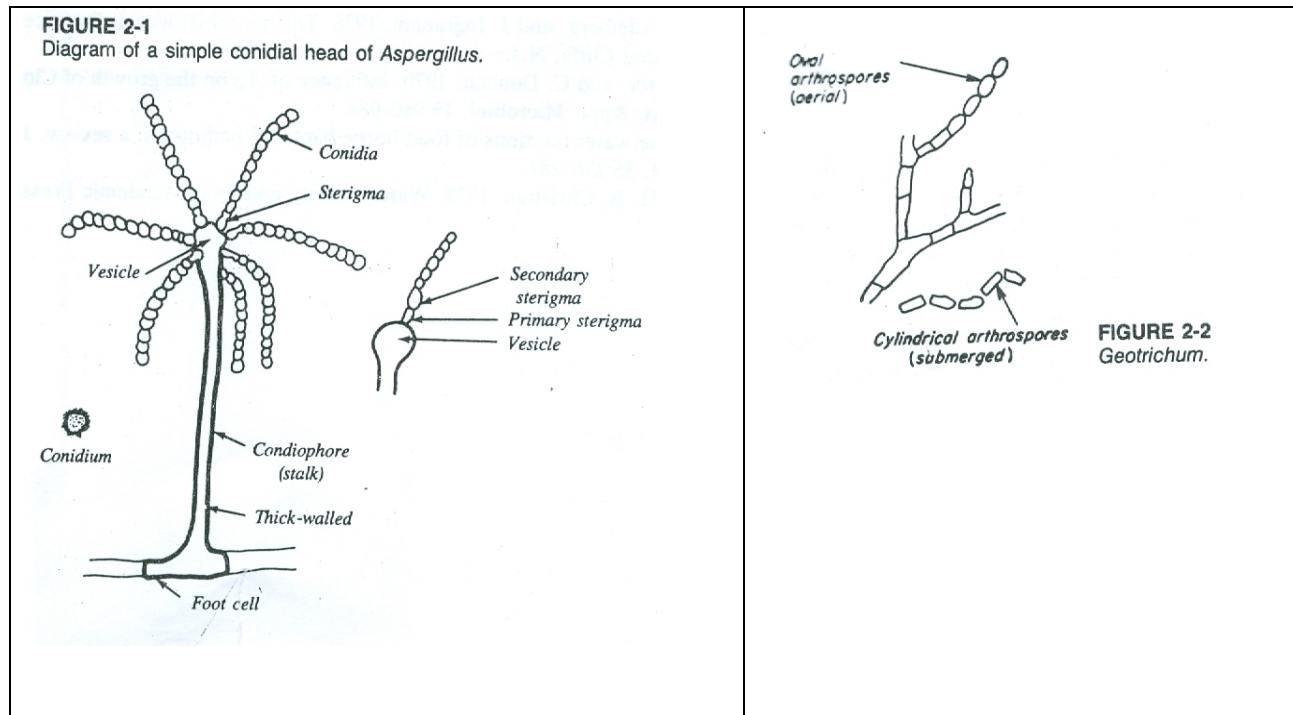


FIGURE 2-3
Rhizopus.



Sexual Spores: The molds which can produce sexual spores are classified on the basis of the manner of formation of these spores and the type produced. The non septate molds (*Phycomycetes*) that produce.

1. Oospores are termed *Oomycetes*. These molds are mostly aquatic; however, included in this group are several important plant pathogens. The oospores are formed by the union of a small male gamete and a large female gamete.

2. Zygosporangia: *Zygomycetes* form zygosporangia by the union of the tips of two hyphae which often appear similar and which may come from the same mycelium or from different mycelia.

Both Oospores and zygosporangia are covered by a tough wall and can survive drying for long periods.

3. Ascospores: The *Ascomycetes* (septate) form sexual spores known as ascospores, which are formed after the union of two cells from the same mycelium or from two separate mycelia. The ascospores, resulting from cell division after conjugation, are in an ascus, or sac, with usual eight spores per ascus.

4. Basidiospores: The *Basidiomycetes*, which include most mushrooms, plant rusts, smuts, etc., form a fourth type of sexual spore, the basidiospore.

Cultural Characteristics

Some molds are loose and fluffy; others are compact. Some look velvety on the upper surface, some dry and powdery, and others wet or gelatinous. Definite zones of growth in the thallus distinguish some molds, e.g., *Aspergillus niger*.

Pigments in the mycelium-red, purple, yellow, brown, gray, black, etc - are characteristic, as are the pigments of masses of asexual spores; green, blue-green, yellow, orange, pink, lavender, brown, gray, black, etc. The appearance of the reverse side of a mold on an agar plate may be striking, like the opalescent blue-black or greenish-black color of the underside of *Cladosporium*.

Physiological characteristics:

The physiological characteristics of molds will be discussed briefly.

Moisture Requirements In general most molds require less available moisture than do most yeasts and bacteria. An approximate limiting total moisture content of a given food for mold growth can be estimated, and therefore it has been claimed that below 14 to 15 percent total moisture in flour or some dried fruits will prevent or greatly delay mold growth.

Temperature Requirements Most molds would be considered mesophilic i.e., able to grow well at ordinary temperatures. The optimal temperature for most molds is around 25 to 30⁰ C, but some grow well at 35 to 37⁰C or above, e.g., *Aspergillus spp.*, and some at still higher temperatures. A number of molds are psychrotrophic; i.e., they grow fairly well at temperatures of refrigeration, and some can grow slowly at temperatures below freezing. Growth has been reported at as low as - 5 to - 10⁰C. A few are thermophilic; i.e., they have a high optimal temperature.

Oxygen and pH Requirements Molds are aerobic; i.e., they require oxygen for growth; this is true at least for the molds growing on foods. Most molds can grow over a wide range of hydrogen-ion concentration (pH 2 to 8.5), but the majority are favored by an acid pH.

Food Requirements Molds in general can utilize many kinds of foods, ranging from simple to complex. Most of the common molds possess a variety of hydrolytic enzymes, and some are grown for their amylases, pectinases, proteinases, and lipases.

Inhibitors Compounds inhibitory to other organisms are produced by some molds, such as *penicillin* from *Penicillium chrysogenum* and *clavacin* from *Aspergillus clavatus*. Certain chemical compounds are mycostatic, inhibiting the growth of molds (sorbic acid, propionates, and acetates are examples), or are specifically fungicidal, killing molds.

Classification and identification of molds

Molds are plants of the kingdom Myceteae. They have no roots, stems, or leaves and are devoid of chlorophyll. They belong to the Eumycetes, or true fungi, and are subdivided further to subdivisions, classes, orders, families, and genera.

The following criteria are used chiefly for differentiation and identification of molds:

- 1 Hyphae septate or non-septate
- 2 Mycelium clear or dark (smoky)
- 3 Mycelium colored or colorless
- 4 Whether sexual spores are produced and the type: oospores, zygospores, or ascospores
- 6 Characteristics of the spore head
 - a) Sporangia: size, color, shape, and location
 - b) Spore heads bearing conidia: single conidia, chains, budding conidia, or masses; shape and arrangement of sterigmata or phialides; gumming together of conidia
- 7 Appearance of sporangiophores or conidiophores: simple or branched, and if branched the type of branching; size and shape of columella at tip of sporangiophore; whether conidiophores are single or in bundles
- 8 Microscopic appearances of the asexual spores, especially of conidia: shape, size, color; smooth or rough; one-, two-, or many-celled
- 9 Presence of special structures (or spores): stolons, rhizoids, foot cells, apo-physis, chlamydospores, sclerotia, etc.

Molds of Industrial Importance

Mucor: *Mucor* are involved in the spoilage of some foods and the manufacture of others. A widely distributed species is *M. racemosus*; *M. rouxii* is used in the "Amylo" process for the saccharification of starch, and mucors help ripen some cheese, (e.g., Gammelost) and are used in making certain Oriental foods.

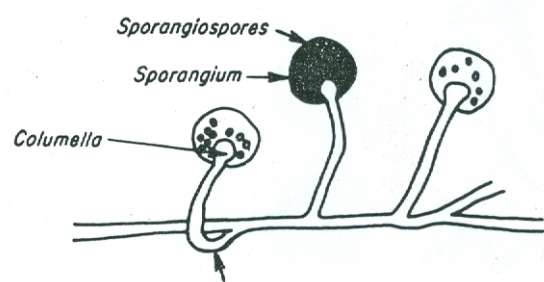


FIGURE 2-4
Mucor.

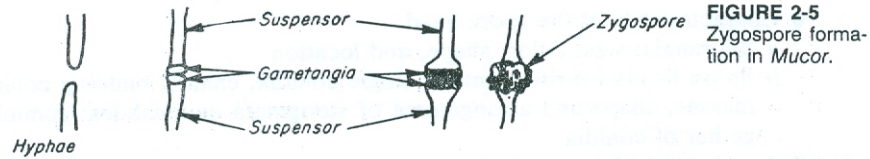


FIGURE 2-5
Zygospore formation in *Mucor*.

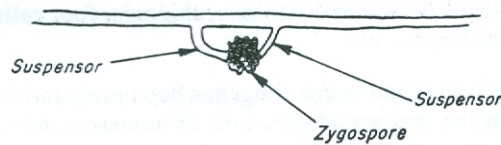


FIGURE 2-6
Zygospore formation in *Zygorrhynchus*.

FIGURE 2-8
Diagram of *Absidia*, showing locations of sporangiophores and rhizoids.

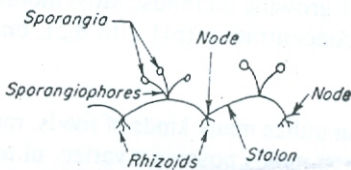
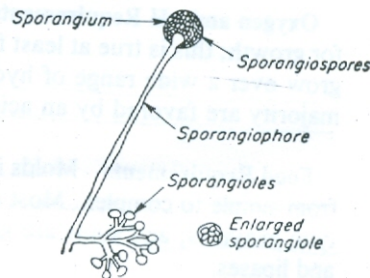


FIGURE 2-9
Thamnidium.



Zygorrhynchus These soil molds are similar to *Mucor* except that the zygo-spore suspensors are markedly unequal in size.

Rhizopus *Rhizopus stolonifer*, the so-called bread mold, is very common and is involved in the spoilage of many foods: berries, fruits, vegetables, bread, etc.

Absidia: Similar to *Rhizopus*, except that sporangia are small and pear-shaped.

Thamnidium: *Thamnidium elegans* is found on meat in chilling storage, causing "whiskers" on the meat.

Aspergillus: The aspergilli are very widespread. Many are involved in the spoilage of foods, and some are useful in the preparation of certain foods. The molds grow well in high concentrations of sugar and salt and hence in many foods of low moisture content. Conidia of this group are some shade of green. *Eurotium*, a name reserved for members having a perfect (sexual) stage.

The *A. niger* group, with *A. niger* as a leading species, is widespread and may be important in foods. The spore-bearing heads are large, tightly packed, and globular and may be black, brownish-black, or purple-brown.

The *A. flavus-oryzae* group includes molds important in the making of some Oriental foods and the production of enzymes. Conidia give various yellow to green shades to the spore heads, and dark sclerotia may be formed.

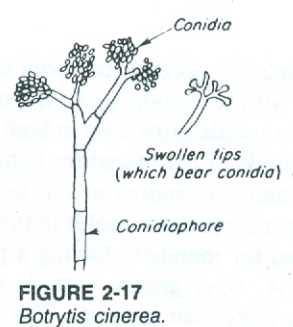
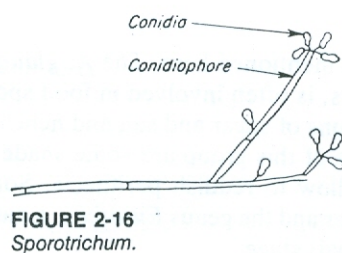
***Penicillium*:** This is another genus that is widespread in occurrence and important in foods. The genus is divided into groups and subgroups, and there are numerous species. The genus is divided into large groups on the basis of the branching of the spore-bearing heads, or penicilli (little brushes). These heads, or verticillata, are a whorl or cluster of three or more elements: sterigmata, metulae (subbranches), and branches.

P. expansum, the blue-green-spored mold, causes soft rots of fruits. Other important species are *P. digitatum*, with olive, or yellowish-green conidia, causing a soft rot of citrus fruits; *P. italicum*, called the "blue contact mold" with blue green conidia, also rotting citrus fruit; *P. camemberti*, with grayish conidia, useful in the ripening of Camembert cheese; and *P. roqueforti*, with bluish-green conidia, aiding in the ripening of blue cheeses, e.g., *Roquefort*.

Trichothecium The common species, *T. roseum* (Figure 2-14), is a pink mold which grows on wood, paper, fruits such as apples and peaches, and vegetables such as cucumbers and cantaloupes.

Geotrichum (Oospora or Oidium) This genus is included with the yeast like fungi by some writers and with the molds by others. Species may be white, yellowish, orange, or red, with the growth appearing first as a firm, felt like mass that later becomes soft and creamy. *Geotrichum candidum* (*Oospora lactis*), often called the "dairy mold," gives white to cream-colored growth.

***Neurospora (Monilia)*:** This genus has been described under various names because of the confusion concerning its classification. It is classed among the perfect molds (producing sexual spores) and call the genus *Neurospora*. *Neurospora (Monilia) sitophila*, the most important species in foods, sometimes is termed the "red bread mold" because its pink, loose-textured growth often occurs on bread.



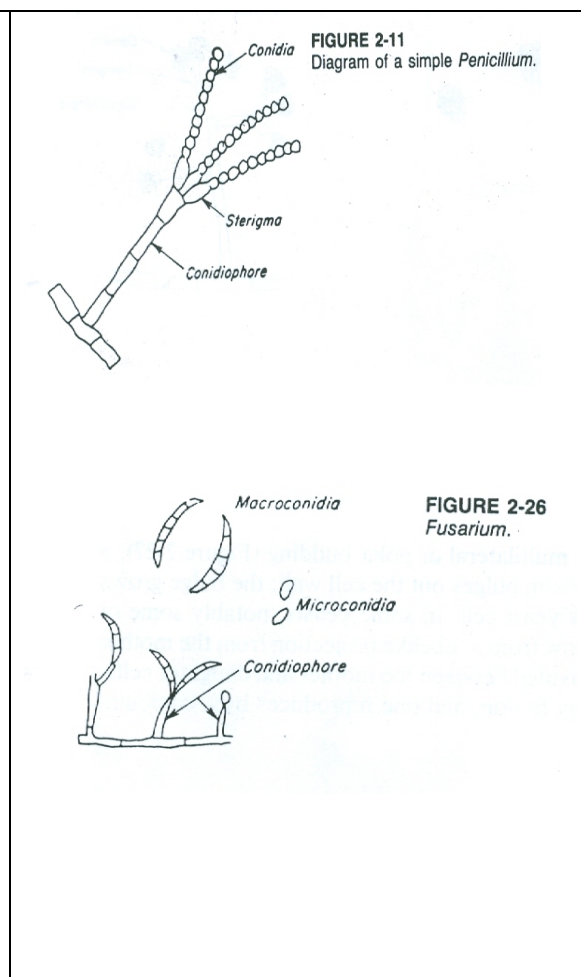
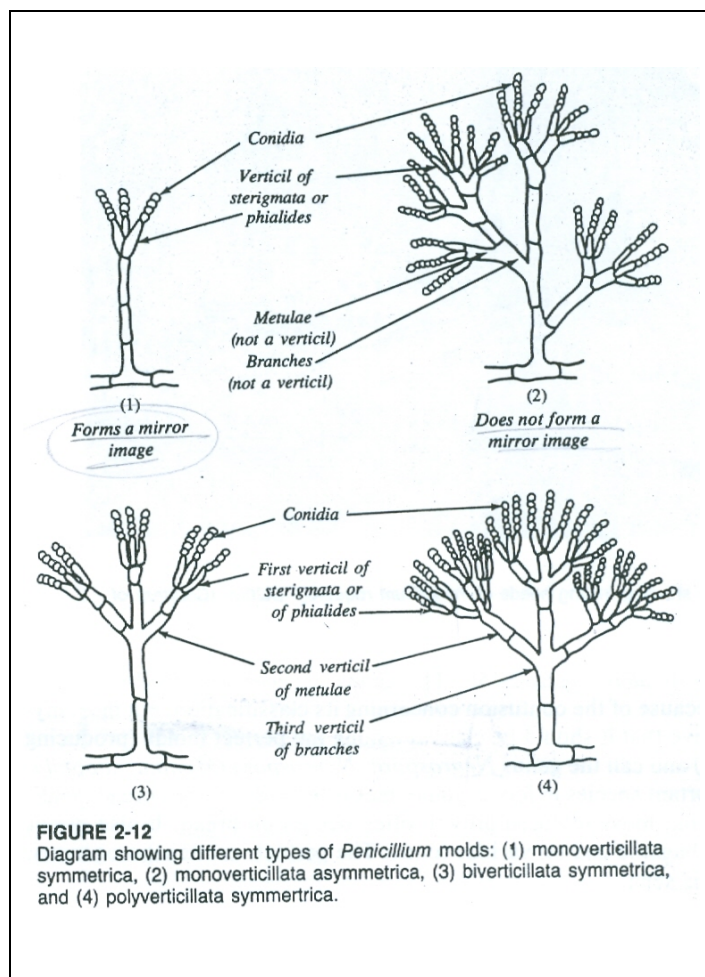


FIGURE 2-14
Trichothecium.

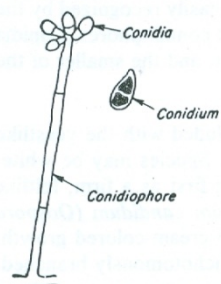


FIGURE 2-15
Neurospora (Monilia).

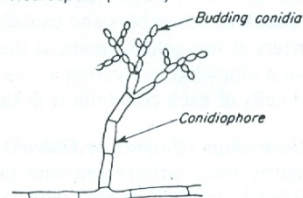


FIGURE 2-18
Cephalosporium.

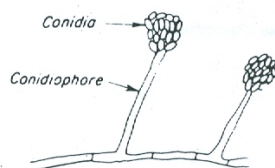
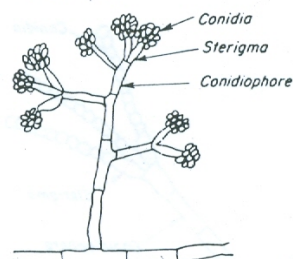


FIGURE 2-19
Trichoderma.



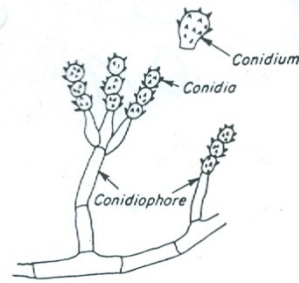


FIGURE 2-20
Scopulariopsis.

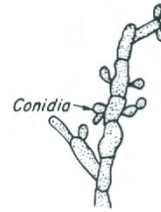


FIGURE 2-21
Pullularia.

FIGURE 2-22
Cladosporium.

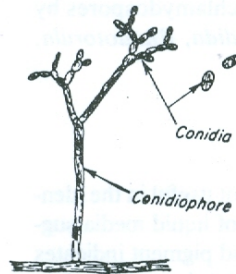


FIGURE 2-23
Helminthosporium.

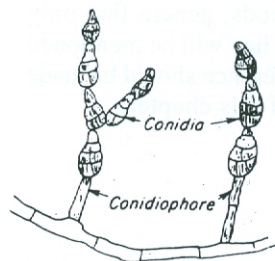
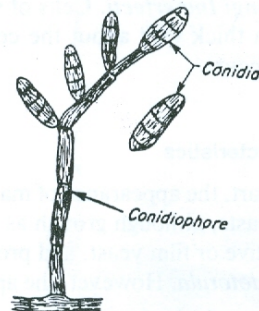


FIGURE 2-24
Alternaria.

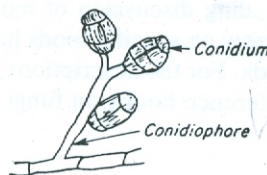


FIGURE 2-25
Stemphylium.

Sporotrichum Among the saprophytic species is *S. carnis*, found growing on chilled meats, where it causes "white spot."

Botrytis One species important in foods is *B. cinerea*. It causes a disease of grapes but may grow saprophytically on many foods.

Cephalosporium: *Cephalosporium acremonium* is a common species.

Trichoderma *T. viride* is a common species. The mature mold plant is bright green because the balls of green conidia are glued together, and tufts of white hyphae (sterile) stick up well above the conidiophores.

Scopulariopsis *S. brevicaulis* is common species. Colonies are brownish and cottony.

***Pullularia*:** Ovate, hyaline conidia (blastospores or buds from preexisting cells) borne as lateral buds on all parts of the mycelium. Colonies are pale and slimy and yeastlike when young, becoming mycelial and dark and leathery in age. *P. pullulans* is a common species.

Cladosporium *C. herbarum* is a leading species. These dark molds cause "black spot" on a number of foods, on cellar walls, etc. Colonies of *C. herbarum* are restricted in growth and are thick, velvety, and olive - to gray-green; the reverse side of the plant is a striking opalescent blue-black to greenish-black.

Helminthosporium Species of this genus are for the most part plant pathogens but may grow saprophytically on vegetable materials

Alternaria Molds of this genus are common causes of the spoilage of foods. *A. citri* (rotting citrus fruits), *A. tenuis*, and *A. brassicae* common species.

***Stemphylium*:** This, too, is a common genus, The conidia are dark and multicellular but have, fewer cross - walls than those of *Alternaria* and are rounded at both ends.

Fusarium Molds of this genus often grow on foods. The species are very difficult to identify, and the appearance of growth is variable.

Endomyces Yeast like fungi, forming mycelium and arthrospores. Some species rot fruits.

Monascus Colonies of *M. purpureus* are thin and spreading and reddish or purple in color. Found on dairy products and on Chinese red rice (ang -khak).

Sclerotinia Some species cause rots of vegetables and fruits, where they are present in the conidial stage. The lemon-shaped conidia are in chains, with a "plug" separating conidia.

LECTURE-6

Yeasts and Yeast like fungi – General characteristics of yeasts, classification and identification of yeasts, yeasts of industrial importance

Yeasts and yeast like fungi

Like mold, the term "yeast" is commonly used but hard to define. It refers to those fungi which are generally not filamentous but unicellular and ovoid or spheroid and which reproduce by budding or fission.

Yeasts may be useful or harmful in foods. Yeast fermentations are involved in the manufacture of foods such as bread, beer, wines, vinegar, and surface ripened cheese, and yeasts are grown for enzymes and for food. Yeasts are undesirable when they cause spoilage of sauerkraut, fruit juices, syrups; molasses, honey, jellies, meats, wine, beer, and other foods.

General characteristics of yeasts

Yeasts are classified chiefly on their morphological characteristics, although their physiological ones are more important to the food microbiologist.

Morphological Characteristics

The morphological characteristics of yeasts are determined by microscopic examination.

Form and Structure The form of yeasts may be spherical to ovoid, lemon shaped, pear-shaped, cylindrical, triangular, or even elongated into a false or true mycelium. They also differ in size. Visible parts of the structure are the cell wall, cytoplasm, water vacuoles, fat globules, and granules, which may be metachromatic, albuminous, or Starchy. Special staining is necessary to demonstrate the nucleus.

Reproduction

Most yeasts reproduce asexually by multilateral or polar budding, a process in which some of the protoplasm bulges out the cell wall; the bulge grows in size and finally walls off as a new yeast cell. In some yeasts, notably some of the film yeasts, the bud appears to grow from a tube like projection from the mother cell. Replicated nuclear material is divided between the mother and daughter cells. A few species of yeasts reproduce by fission, and one reproduces by a combination of fission and budding.

Sexual reproduction of "true" yeasts (*Ascomycotina*) results in the production of ascospores, the yeast cell serving as the ascus. The formation of ascospores follows conjugation of two cells in most species of true yeasts, but some may produce ascospores without conjugation, followed by conjugation of ascospores or small daughter cells. The usual number of spores per ascus and the appearance of the ascospores are characteristic of the kind of yeast. The

ascospores may differ in color, in smoothness or roughness of their walls, and in their shape (round, oval, reniform, bean- or sickle-shaped, Saturn- or hat~ shaped, hemispherical, angular, fusiform, or needle-shaped).

"False" yeasts, which produce no ascospores or other sexual spores, belong to the *Fungi Imperfecti*. Cells of some yeasts become chlamydospores by formation of a thick wall about the cell, for example, *Candida*, *Rhodotorula*, and *Cryptococcus*.

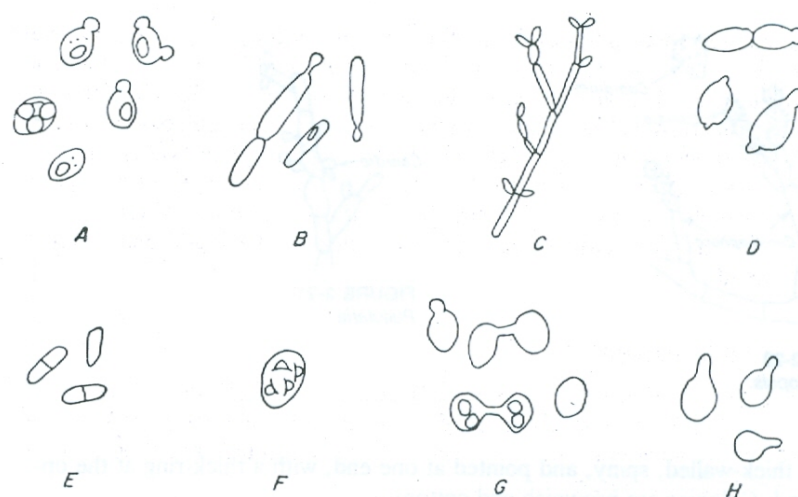


FIGURE 2-27

Yeasts of different shapes: (A) *Saccharomyces cerevisiae*, with budding cells and one ascus with four ascospores, (B) *Candida* yeast, with elongated cells, (C) *Candida*, showing pseudomycelium, (D) apiculate (lemon-shaped) yeast, (E) *Schizosaccharomyces*, multiplying by fission, (F) *Hansenula*, with ascospores shaped like derby hats, (G) *Zygosaccharomyces*, showing conjugation with ascus and four ascospores, (H) flask-shaped yeasts.

Cultural Characteristics

Growth as a film on the surface of liquid media suggests an oxidative or film yeast, and production of a carotenoid pigment indicates the genus *Rhodotorula*. The appearance of the growth is important when it causes colored spots on foods. It is difficult to tell yeast colonies from bacterial ones on agar plates; the only certain way is by means of microscopic examination of the organisms. Most young yeast colonies are moist and somewhat slimy but may appear mealy; most colonies are whitish, but some are cream-colored or pink. Some colonies change little with age, but others become dry and wrinkled.

Yeasts are oxidative, fermentative, or both. The oxidative yeasts may grow as a film, pellicle, or scum on the surface of a liquid and then are termed film yeasts. Fermentative yeasts usually grow throughout the liquid and produce carbon dioxide.

Physiological Characteristics

Most common yeasts grow best with a plentiful supply of available moisture. But since many yeasts grow in the presence of greater concentrations of solutes (such as sugar or salt) than most bacteria.

Most yeast requires more moisture than molds, however. on the basis of water activity or a_w , yeasts may be classified as ordinary if they do not grow in high concentrations of solutes, i.e., in a low a_w , and as osmophilic if they do.

Lower limits of a_w for ordinary yeasts range from 0.88 to 0.94.

Osmophilic yeasts have been found growing slowly in media with an a_w as low as 0.62 to 0.65 in syrups, although some osmophilic yeasts are stopped at about 0.78 in both salt brine and sugar syrup.

The a_w values will vary with the nutritive properties of the substrate, pH, temperature, availability of oxygen, and presence or absence of inhibitory substances.

The range of temperature for growth of most yeasts is 25 to 30°C and the maximum about 35 to 47°C. Some kinds can grow at 0°C or less. The growth of most yeasts is favored by an acid reaction in the vicinity of pH 4 to 4.5, and they will not grow well in an alkaline medium unless adapted to it. Yeasts grow best under aerobic conditions, but the fermentative types can grow anaerobically, although slowly.

In general, sugars are the best source of energy for yeasts, although oxidative yeasts, e.g., the film yeasts, oxidize organic acids and alcohol. Carbon dioxide produced by bread yeasts accomplishes the leavening of bread, and alcohol made by the fermentative yeasts is the main product in the manufacture of wines, beer, industrial alcohol, and other products. The yeasts also aid in the production of flavors or "bouquet" in wines.

Nitrogenous foods utilized vary from simple compounds such as ammonia and urea to amino acids and polypeptides. In addition, yeasts require accessory growth factors.

Yeasts may change in their physiological characteristics, especially the true, or ascospore-forming, yeasts, which have a sexual method of reproduction. These yeasts can be bred for certain characteristics or may mutate to new forms. Most yeasts can be adapted to conditions which previously would not support good growth. Illustrative of different characteristics within a species is the large number of strains of *Saccharomyces cerevisiae* suited to different uses, e.g., bread strains, beer strains, wine strains, and high-alcohol-producing strains or varieties.

Classification and identification of yeasts

The true yeasts are in the subdivision *Ascomycotina*, and the false, or asporogenous, yeasts are in the subdivision *Fungi Imperfecti* or *Deuteromycotina*. Certain yeasts are actually represented in two different genera based on whether they reproduce sexually.

The principal bases for the identification and classification of genera of yeasts are as follows:

- 1 Whether ascospores are formed.
- 2 If they are spore-forming:
 - a) The method of production of ascospores:
 - (1) Produced without conjugation of yeast cells (parthenogenetically). Spore formation may be followed by
 - (a) Conjugation of ascospores. (b) Conjugation of small daughter cells.
 - (2) Produced after isogamic conjugation (conjugating cells appear similar).
 - (3) Produced by heterogamic conjugation (conjugating cells differ in appearance).
 - b) Appearance of ascospores: shape, size, and color. Most spores are spheroidal or ovoid, but some have odd shapes, e.g., most species of *Hansenula*, which look like derby hats
 - c) The usual number of ascospores per ascus: one, two, four, or eight.
- 3 Appearance of vegetative cells: shape, size, color, inclusions.
- 4 Method of asexual reproduction:
 - a. Budding. b. Fission. c. Combined budding and fission. d. Arthrospores (oidia).
5. Production of a mycelium, pseudo mycelium, or no mycelium.
6. Growth as a film over surface of a liquid (film yeasts) or growth throughout medium.
- 7 Color of macroscopic growth.
- 8 Physiological characteristics (used primarily to differentiate species or strains within a species):
 - a. Nitrogen and carbon sources. b. Vitamin requirements. c. Oxidative or fermentative: film yeasts are oxidative; other yeasts may be fermentative or fermentative and oxidative.
 - d. Lipolysis; urease activity, acid production, or formation of starch like compounds.

Yeasts of industrial importance

Most yeasts used industrially are in the genus *Saccharomyces*. The term "wild yeast" is applied to any yeast other than the one being used or encouraged. Thus yeast employed in one process could be a wild yeast in another. Most of the troublesome wild yeasts are asporogenous, or false, yeasts.

Genus *Schizosaccharomyces* These yeasts, which reproduce asexually by fission and form four or eight ascospores per ascus after isogamic conjugation, have been found in tropical fruits, molasses, soil, honey, and elsewhere. A common species is *S. pombe*.

Genus *Saccharomyces* Cells of these yeasts may be round, ovate, or elongated and may form a pseudo-mycelium. Reproduction is by multipolar budding or by ascospore formation. The ascospores, one to four per ascus, are usually round or ovate. The leading species, *S. cerevisiae*, is employed in many food industries, with special strains used for the leavening of bread, as top yeasts for ale, for wines, and for the production of alcohol, glycerol, and invertase.

Top yeasts are very active fermenters and grow rapidly at 20°C. The clumping of the cells and the rapid evolution of CO₂ sweep the cells to the surface, hence the term top yeast.

Bottom yeast do not clump, grow more slowly, and are best fermenters at lower temperatures (10 to 15°C). The absence of clumping and the slower growth and evolution of CO₂ permit the yeast to settle to the bottom, hence the term bottom yeast. These characteristics of brewers' yeast are observations and do not explain why some yeast clump, or flocculate.

S. cerevisiae var. *ellipsoideus* is a high-alcohol-yielding variety used to produce industrial alcohol, wines, and distilled liquors. *S. uvarum*, a bottom yeast, is used in making beer. *S. fragilis* and *S. Lactis*, because of their ability to ferment lactose, may be important in milk or milk products. *S. rouxii* and *S. mellis* are osmophilic.

Many of the *Saccharomyces* have been reclassified. For example, *S. uvarum* is now considered a variant of *S. cerevisiae*, *S. fragilis* is now *Kluyveromyces marxianus*, and *S. lactis* is now *K. marxianus* var. *lactis*.

S. rouxii, *S. mellis*, and *S. nussbaumeri* are now *Zygosaccharomyces rouxii*. *Debaryomyces hansenii* is now *D. hansenii*.

Genus *Kluyveromyces*. These yeasts reproduce by multilateral budding, and ascospores are liberated upon maturity.

Genus *Zygosaccharomyces*. These yeasts are notable for their ability to grow in high concentrations of sugar (hence they are termed osmophilic) and are involved in the spoilage of honey, sirups, and molasses and in the fermentation of soy sauce and some wines. *Zygosaccharomyces nussbaumeri* grows in honey.

Genus *Pichia* These oval to cylindrical yeasts may form pseudomycelia.

Ascospores are round or hat-shaped, and there are one to four per ascus. A pellicle is formed on liquids; e.g., *P. membranaefaciens* grows a pellicle on beers or wines.

Genus *Hansenula* These yeasts resemble *Pichia* in appearance but are usually more fermentative, although some species form pellicles. Ascospores are hat- or Saturn-shaped.

Genus *Debaryomyces* These round or oval yeasts form pellicles on meat brines. Ascospores have a warty surface. *D. kloeckeri* grows on cheese and sausage.

Genus *Hanseniaspora* These lemon-shaped (apiculate) yeasts grow in fruit juices. *Nadsonia* yeasts are large and lemon-shaped.

False Yeasts (Fungi Imperfect)

Genus *Torulopsis* These round to oval fermentative yeasts with multilateral budding cause trouble in breweries and spoil various foods. *T. sphaerica* ferments lactose and may spoil milk products. Other species can spoil sweetened condensed milk, fruit-juice concentrates, and acid foods.

Genus *Candida* These yeasts form pseudohyphae or true hyphae, with abundant budding cells or blastospores, and may form chlamydospores. Many form films and can spoil foods high in acid and salt. *C. utilis* is grown for food and feed. *C. krusei* has been grown with dairy starter cultures to maintain the activity and increase the longevity of the lactic acid bacteria. Lipolytic *C. lipolytica* can spoil butter and oleomargarine.

Genus *Brettanomyces* These ogive - or arch-shaped yeasts produce high amounts of acid and are involved in the late fermentation of Belgian lambic beer and English beers. They also are found in French wines. *B. bruxellensis* and *B. lambicus* are typical species.

Genus *Kloeckera* These are imperfect apiculate or lemon-shaped yeasts. *K. apiculata* is common on fruits and flowers and in the soil.

Genus *Trichosporon* These yeasts bud and form arthrospores. They grow best at low temperatures and are found in breweries and on chilled beef. *T. pullulans* is a common species.

Genus *Rhodotorula* These red, pink, or yellow yeasts may cause discolorations on foods, e.g., colored spots on meats or pink areas in sauerkraut.

Groups of Yeasts

Film yeasts, in the genera *Pichia*, *Hansenula*, *Debaryomyces*, *Candida*, and *Trichosporon*, grow on the surface of acid products such as sauerkraut and pickles, oxidize the organic acids, and enable less acid-tolerant organisms to continue the spoilage. *Hansenula* and *Pichia* tolerate high levels of alcohol and may oxidize it in alcoholic beverages. *Pichia* species are encouraged to grow on Jerez and Arbois wine, to which they are supposed to impart distinctive flavors and esters. *Debaryomyces* is very salt tolerant and can grow on cheese brines with as much as 24 percent salt. The film yeasts produce little or no alcohol from sugars.

Apiculate or lemon-shaped yeasts, in *Saccharomyces*, *Hanseniaspora*, *Nadsonia*, and *Kloeckera*, are considered objectionable in wine fermentations because they give off-flavors, low yields of alcohol, and highly volatile acid.

Osmophilic yeasts (*Saccharomyces rouxii* and *S. mellis*) grow well in an environment of high osmotic pressure, i.e., in high concentrations of sugars, salts, or other solutes, causing spoilage of dry fruits, concentrated fruit juices, honey, maple sirup, and other high-sugar solutions.

Salt-tolerant yeasts grow in curing brines, salted meats and fish, soy sauce, miso paste, and tamari sauce. The most salt-tolerant of the film yeasts are species of *Debaryomyces*, which grow on curing brines and on meats and cucumbers in them, as does *Saccharomyces rouxii*, which can grow as a film on brine. Yeasts in various other genera (*Torulopsis*, *Brettanomyces*, and others) also grow in brines. Yeasts grow in soy sauce with its high content of salt (about 18 percent). *Saccharomyces rouxii* is considered of great importance in the production of alcohol and flavor, but species of *Torulopsis*, *Pichia*, *Candida*, and *Trichosporon* also may grow. Film-forming *S. rouxii* and *Pichia* are sometimes involved in the spoilage of soy sauce. Similar yeasts are involved in miso production, but kinds will vary as the salt concentration is varied between 7 and 20 percent.

LECTURE-7

Bacteria – Morphological characteristics important in Food Bacteriology. Cultural and Physiological characteristics important in food bacteriology. Genera of bacteria important in Food Bacteriology groups of bacteria important in food bacteriology

Bacteria

Morphological characteristics important in food bacteriology

One of the first steps in the identification of bacteria in a food is microscopic examination to ascertain the shape, size, aggregation, structure, and staining reactions of the bacteria present. The following characteristics may be of special significance.

Encapsulation

The presence of capsules or slime may account for sliminess or ropiness of a food. In addition, capsules serve to increase the resistance of bacteria to adverse conditions, such as heat or chemicals. To the organism they may serve as a source of reserved nutrients. Most capsules are polysaccharides of dextrin, dextran, or levan.

Formation of Endospores

Bacteria of the genera *Bacillus*, *Clostridium*, *Desulfotomaculum*, *Sporolactobacillus* (rods), and *Sporosarcina* (cocci) share the ability to form endospores.

Bacillus - aerobic and some facultative anaerobic and *Clostridium* - anaerobic.

Endospores are formed at an intracellular site, are very refractile, and are resistant to heat, ultraviolet light, and desiccation.

Formation of Cell Aggregates

It is characteristic of some bacteria to form long chains and of others to clump under certain conditions. It is more difficult to kill all bacteria in intertwined chains or sizable clumps than to destroy separate cells.

Cultural characteristics important in food bacteriology:

Bacterial growth in and on foods often is extensive. Pigmented bacteria cause discolorations on the surfaces of foods; films may cover the surfaces of liquids; growth may make surfaces slimy; or growth throughout the liquids may result in undesirable cloudiness or sediment.

Physiological characteristics important in food bacteriology

These changes include hydrolysis of complex carbohydrates to simple ones; hydrolysis of proteins to polypeptides, amino acids, and ammonia or amines; and hydrolysis of fats to glycerol and fatty acids. O-R reactions, which are utilized by the bacteria to obtain energy from foods

(carbohydrates, other carbon compounds, simple nitrogen-carbon compounds, etc.), yield such products as organic acids, alcohols, aldehydes, ketones, and gases.

Genera of bacteria important in food bacteriology

The classification given in Bergey's Manual of Systematic Bacteriology, vols. I and II, 1984 and 1986, will be followed.

Genus *Acetobacter* These bacteria oxidize ethyl alcohol to acetic acid. They are rod-shaped and motile and are found on fruits, vegetables, souring fruits, and alcoholic beverages. They are a definite spoilage problem in alcoholic beverages.

Genus *Aeromonas* These are gram-negative rods with an optimum temperature for growth of 22 to 28 C. They are facultative anaerobes and can be psychrophilic. They are frequently isolated from aquatic environments. *A. hydrophila* can be a human pathogen; it is also pathogenic to fish, frogs, and other mammals.

Genus *Alcaligenes* As the name suggests, an alkaline reaction usually is produced in the medium of growth. *A. viscolactis* causes ropiness in milk, and *A. metalcaligenes* gives a slimy growth on cottage cheese. These organisms come from manure, feeds, soil, water, and dust. This genus also contains organisms which were formerly classified in the genus *Achromobacter*.

Genus *Alteromonas* Several former species of *Pseudomonas* are now classified as *Alteromonas*. They are marine organisms that are potentially important in sea foods.

Genus *Arthrobacter* A predominant soil organism, it is inert in most foods. However, some species can grow at 5⁰ C and would be considered psychrotrophs.

Genus *Bacillus* The endospores of species of this aerobic to facultative genus usually do not swell the rods in which they are formed. Different species may be mesophilic or thermophilic, actively proteolytic, moderately proteolytic, or non proteolytic, gas-forming or not, and lipolytic or not. In general the spores of the mesophiles, e.g., *B. subtilis*, are less heat-resistant than spores of the thermophiles. Spores of the obligate thermophiles, e.g., *B. stearothermophilus*, are more resistant than those of facultative thermophiles, e.g., *B. coagulans*. The actively proteolytic species usually may also sweet-curdle milk; *B. cereus* is such a species. The two chief acid- and gas-forming species, *B. polymyxa* and *B. macerans*, sometimes are termed "aerobacilli." Many of the mesophiles can form acid from glucose or other sugar but usually form only a small amount that often is neutralized by ammonia produced from the nitrogenous food. The thermophilic flat sour bacteria that spoil canned vegetables can produce considerable amounts of lactic acid from sugar, and such a culture, e.g., *B. coagulans*, may be employed for the manufacture of lactic acid. The soil is an important source of *Bacillus* species.

Genus *Brevibacterium* *B. linens* is related to *Arthrobacter globiformis* and may be synonymous. *B. linens* may be important in the surface smear of certain cheeses, e.g., brick or Limburger, where the culture produces an orange red pigmentation and helps ripening.

Genus *Brochotrix* These are gram-positive rods which can form long filamentous like chains that may fold into knotted masses. The optimum temperature for growth is 20 to 25°C, but growth can occur over a temperature range of 0 to 45°C depending on the strain. They can spoil a wide variety of meats and meat products when they are stored aerobically or vacuum packed and held refrigerated. *B. thermosphacta* is the only species listed.

Genus *Campylobacter* These bacteria were originally classified in the genus vibrio. Several strains of *C. fetus subsp. jejuni* have been associated with gastroenteritis in humans.

Genus *Clostridium* The endospores of species of this genus of anaerobic to microaerophilic bacteria usually swell the end or middle of the rods in which they are formed. Different species may be mesophilic or thermophilic and proteolytic or non-proteolytic. *Clostridium thermosaccharolyticum* is an example of a saccharolytic obligate thermophile; this organism causes gaseous spoilage of canned vegetables. Putrefaction of foods often is caused by mesophilic, proteolytic species, such as *C. lentoputrescens* and *C. putrefaciens*. The violent disruption of the curd in milk by *C. perfringens* or similar species results in a "stormy fermentation" of milk, and the lactate fermenting *C. butyricum* is a cause of late gas in cured cheese. The soil is the primary source of *Clostridium* spp., although they also may come from bad silage, feeds, and manure.

Genus *Corynebacterium* The diphtheria organism, *C. diphtheriae*, may be transported by foods. *C. bovis*, with the slender, barred, or clubbed rods characteristic of the genus, is commensal on the cow's udder, can be found in aseptically drawn milk, and may be a cause of bovine mastitis.

Genus *Dessulfotomaculum* A gram, negative rod which swells when an endospore forms. They are common inhabitants of the soil, fresh water, and the rumen. Sulfur compounds can serve as the terminal electron acceptor in respiration and thereby be reduced to hydrogen sulfide. *Clostridium nigrificans*, which is responsible for sulfide stinker spoilage in canned foods, is now called *D. nigrificans*.

Genus *Enterobacter* Some were formerly classified as *Aerobacter*. They are widely distributed in nature; a member of the coliform group.

Genus *Erwinia* The species of this genus are plant pathogens that cause necrosis, galls, wilts, or soft rots in plants and therefore damage the plants and vegetable and fruit products from them. *E. carotovora* is associated with the market disease called "bacterial soft rot." *E. carotovora subsp.*

carotovora causes rotting in a large number of plants. *E. carotovora subsp. atroseptica* produces a black rot in potatoes. *E. carotovora subsp. betavascularum* causes soft rot in sugar beets.

Genus *Escherichia* Found in feces, a predominant gram-negative rod isolated from the intestinal tract of warm-blooded animals and widely distributed in nature. One of the "coliform group," the genus is divided into many biotypes and serotypes, some of which can be pathogenic to humans.

Genus *Flavobacterium* The yellow to orange-pigmented species of this genus may cause discolorations on the surface of meats and be involved in the spoilage of shellfish, poultry, eggs, butter, and milk. Some of the organisms are psychrotrophic and have been found growing on thawing vegetables.

Genus *Gluconobacter* (Formerly *Acetomonas*). Species can oxidize ethanol to acetic acid. *G. oxydans* causes ropiness in beer following viscous growth in beer or wort.

Genus *Halobacterium* Bacteria of this genus, e.g., *H. salinarium*, are obligate halophiles and are usually chromogenic. They may grow and cause discolorations on foods high in salt, such as salted fish.

Genus *Klebsiella* Many are capsulated. Commonly associated with the respiratory and intestinal tracts of humans. *K. pneumoniae* is the causative organism for a bacterial pneumonia in humans.

Family Lactobacillaceae

Genus *Lactobacillus* The lactobacilli are rods, usually long and slender, that form chains in most species. They are microaerophilic, (some strict anaerobes are known), are catalase-negative and gram-positive, and ferment sugars to yield lactic acid as the main product.

Homofermentation: They ferment sugar chiefly to lactic acid if they are homo-fermentative, with small amounts of acetic acid, carbon dioxide, and trace products. The homo-fermentative lactobacilli with optimal temperatures of 37 C or above include *L. bulgaricus**, *L. helveticus*, *L. lactis**, *L. acidophilus*, *L. thermophilus*. The homo-fermentative lactobacilli with lower optimal temperatures include *L. casei*, *L. plantarum*, and *L. leichmannii**

Heterofermentation: If they are hetero-fermentative, they produce appreciable amounts of volatile products, including alcohol, in addition to lactic acid. *L. delbrueckii*. *L. fermentum* is the chief example of a hetero-fermentative lactobacillus growing well at higher temperatures. Hetero-fermentative species grow at lower temperatures are *L. brevis*, *L. buchneri*, *L. pastorianus**, *L. hilgardii*, and *L. trichodes**.

All the above species except *L. delbrueckii*, *L. leichmannii*, *L. hilgardii*, *L. trichodes**, and some strains of *L. brevis* ferment lactose with the production of lactic acid and therefore may

be of importance in the dairy industries. Chief sources of the lacto-bacilli are plant surfaces, manure, and dairy products.

Characteristics that make the lactobacilli important in foods are

- (1) their ability to ferment sugars with the production of considerable amounts of lactic acid, making it possible to use them in the production of fermented plant and dairy products or the manufacture of industrial lactic acid but resulting in the deterioration of some products, e.g., wine or beer,
- (2) Production of gas and other volatile products by hetero fermentative species, sometimes with damage to the quality of food, as with *L. fermentum* growing in Swiss cheese or *L. hilgardii* or *L. trichodes** in wines.
- (3) Their inability to synthesize most of the vitamins they require, making them unable to grow well in foods poor in vitamins but useful in assays for the vitamin content of foods.
- (4) The heat resistance, or thermoduric properties, of most of the high-temperature lactobacilli, enabling them to survive pasteurization or other heating processes, such as that given the curd in the manufacture of Swiss and similar cheeses.

Species of *Lactobacillus* different from the ones already mentioned have been found growing in refrigerated meats, but only a few names for these lactobacilli have been suggested, e.g., *L. viridescens* for one causing greening of sausage and *L. salinmandus** for one growing in sausage. These lactobacilli are exceptional because of their ability to grow at low temperatures.

Genus *Leuconostoc* This genus, called *Betacoccus* by Orla-Jensen, contains the hetero-fermentative lactic streptococci, which ferment sugar to lactic acid plus considerable amounts of acetic acid, ethyl alcohol, and carbon dioxide. The ability of *L. dextranicum** and *L. cremoris** to ferment citric acid of milk and produce the desirable flavoring substance diacetyl and to stimulate the lactic streptococci. This led to their inclusion as "lactic starter" for buttermilk, butter, and cheese. The habitat of this genus is the surface of plants.

Some of the characteristics of *Leuconostoc* species that make them important in foods are

- (1) Production of diacetyl and other flavoring products,
- (2) Tolerance of salt concentrations, e.g., in sauerkraut and dill-pickle fermentations, permitting *L. mesenteroides* to carry on the first part of the lactic fermentation,
- (3) Ability to initiate fermentation in vegetable products more rapidly than other lactics or other competing bacteria and to produce enough acid to inhibit nonlactics,
- (4) Tolerance of high sugar concentrations (up to 55 to 60 percent for *L. mesenteroides*), permitting the organism to grow in sirups, liquid cake and ice-cream mixes, etc.,

(5) production of considerable amounts of carbon dioxide gas from sugars, leading to undesirable "openness" in some cheeses, spoilage of foods high in sugars (sirups, mixes, etc.), and leavening in some breads,

(6) Heavy slime production in media containing sucrose. This is a desirable characteristic for the production of dextran but a hazard in materials high in sucrose, as in the production of sucrose from sugarcane or beets.

Genus *Listeria* These bacteria are gram-positive, non-spore-forming rods with tumbling motility. *L. monocytogenes* can cause food-borne disease outbreaks.

Genus *Microbacterium* Bacteria of this genus are important because of their resistance to adverse conditions and their use in production of vitamins. They are small, nonmotile, gram-positive, asporogenous, catalase-positive, aerobic, homo-fermentative, lactic acid-producing rods, which sometimes produce palisade arrangements. *M. tacticum* is the species usually encountered. Microbacteria are very resistant to heat for non-spore-forming bacteria, surviving pasteurization of milk readily and even temperatures of 80 to 85° C for 10 min. They therefore are among the thermodurics.

Genus *Micrococcus* Most of the species prominent in foods are gram-positive, aerobic, and catalase-positive. The following characteristics make various groups of micrococci important in foods:

- (1) Some species can utilize ammonium salts or other simple nitrogenous compounds as a sole source of nitrogen,
- (2) Most species can ferment sugars with the production of moderate amounts of acid,
- (3) Some are acid-proteolytic (*M. freudenreichii**),
- (4) some are very salt tolerant and hence able to grow at relatively low levels of available moisture; these grow in meat-curing brines, brine tanks, etc.,
- (5) Many are thermoduric, i.e., survives the pasteurization treatment given market milk (*M. varians*),
- (6) Some are pigmented and discolor the surfaces of foods on which they grow; *M. luteus* is yellow, for example, and *M. roseus* is pink, and
- (7) Some of the micrococci can grow fairly well at temperatures around 10 C or below.

Micrococci are widespread in nature but have been isolated most often from dust and water. They often are found on inadequately cleaned and sanitized food utensils and equipment.

Genus *Mycobacterium* The tubercle bacilli that cause tuberculosis, *M. tuberculosis*, has been spread by foods, especially raw milk from infected cows.

Genus *Pediococcus* The cocci occur singly, in pairs or short chains, or in tetrads (division in two planes) and are gram-positive, catalase-negative, and microaerophilic. They are homo-fermentative. The characteristics that make the organism important in foods have been mentioned: salt tolerance, acid production, and temperature range, especially the ability to grow at cool temperatures. *Pediococci* have been found growing during the fermentation of brined vegetables and have been found responsible for the spoilage of alcoholic beverages, e.g., beer, where their production of diacetyl is undesirable. *P. damnosus* can spoil beer. *P. cerevisiae* has been used as starter culture in fermented sausages.

Genus *Photobacterium* The genus includes coccobacilli and occasional rods which can be luminescent. They are not widespread; however, *P. phosphoreum* has been known to cause phosphorescence of meats and fish.

Genus *Propionibacterium* Members of this genus may be found in foods. In Swiss cheese certain species (e.g., *Propionibacterium freudenreichii*) ferment the lactates to produce the gas that helps form the holes or eyes, and also contribute to the flavor. Pigmented propionic bacteria can cause color defects in cheese.

Genus *Proteus* Bacteria of this genus have been involved in the spoilage of meats, seafood, and eggs. The presence of these bacteria in large numbers in non refrigerated foods has made them suspect as a cause of food poisoning.

Genus *Pseudomonas* A number of species of *Pseudomonas* can cause food spoilage. These bacteria are gram-negative, usually motile, asporogenous rods.

Characteristics of some of the *Pseudomonas* species that make them important in foods are

- (1) Their ability to utilize a large variety of non carbohydrate carbon compounds for energy and their inability to use most carbohydrates.
- (2) Their ability to 'produce a variety of products that affect flavor deleteriously, (3) their ability to use simple nitrogenous foods.
- (4) Their ability to synthesize their own growth factors or vitamins.
- (5) The proteolytic and lipolytic activity of I, some species.
- (6) Their aerobic tendencies, enabling them to grow rapidly and produce oxidized products and slime at the surfaces of foods, where heavy contamination is most likely.
- (7) Their ability to grow well at low (refrigeration) temperatures.
- (8) Pigment production by some species, e.g., the greenish fluorescence by pyoverdine of *Pseudomonas fluorescens* and white, cream-colored, reddish, brown, or even black (*P. nigrificans**) colors of other species.

(9) Their resistance to many disinfectants and sanitizers used in the food industry.

On the other hand, the pseudomonads are limited by a fairly high a_w (0.97 to 0.98), are readily killed by heat, grow poorly if oxygen is not readily available, are not especially resistant to drying, and grow poorly or not at all above 43 °C.

Genus *Salmonella* Species of these enteric pathogens may grow in foods and cause food infections.

Genus *Serratia* Many species produce a pink or magenta pigment and may cause red discolorations on the surface of foods. *S. marcescens* is the most common species.

Genus *Shigella* Species of *Shigella*, causing bacillary dysenteries, may be transported by foods.

Genus *Sporolactobacillus* *Lactobacillus inulinus* has been classified as *S. inulinus* because of its ability to form endospores. It resembles *Lactobacillus* in many characteristics.

Genus *Sporosarcina* A gram-positive coccus that forms endospores. *S. ureae* and *S. halophila* are the two species listed.

Genus *Staphylococcus* The gram-positive staphylococci grow singly, in pairs, in tetrads, or in irregular, grapelike clusters. The most important species, *S. aureus*, usually gives yellow to orange growth, although it may be white on occasion. Many of the beta-hemolytic, coagulase-positive strains are pathogenic, and some produce an enterotoxin which causes food poisoning.

Genus *Streptococcus* The cocci in this genus are characteristically in pairs, in short chains, or in long chains, depending on the species and the conditions of growth, and all are homofermentative. The streptococci important in foods are divided into four groups: the pyogenic, viridans, lactic, and enterococcus groups.

The pyogenic (pus-producing) group pathogenic streptococci, of which *S. agalactiae*, a cause of mastitis in cows, and *S. pyogenes*, a cause of human septic sore throat, scarlet fever, and other diseases are representatives that have been found in raw milk. The pyogenic streptococci cannot grow at 10 or 45°C.

The viridans group includes *S. thermophilus*, a coccus important in cheeses made at high temperatures and yogurt, and *S. bovis* is like *S. thermophilus* and it is thermophilic and therefore counted in the plating of pasteurized milk. These species can grow at 45°C but not at 10°C.

The lactic group contains the important dairy bacteria, *S. lactis* and *S. cremoris**, grow at 10°C but not at 45°C. These bacteria are used as starters for cheese, cultured buttermilk, and some types of butter, along with *Leuconostoc* spp., and *S. lactis* often is concerned in the souring of raw milk.

The enterococcus group consists of *S. faecalis* and *S. faecium*, and some related subspecies. *S. faecalis* is usually the more heat-resistant and comes more from human sources, whereas *S. faecium* has been reported to be more from plant sources. *S. faecalis* subsp. *liquefaciens** is an acid proteolytic variety of *S. faecalis*, and *S. faecalis* subsp. *zymogenes** is a beta-hemolytic variety. Bacteria of this group can grow at both 10 and 45°C.

The enterococci have several characteristics in common that make them unusual streptococci:

- (1) They are thermotolerant, readily surviving the pasteurization treatment of milk or even more heating.
- (2) They tolerate 6.5 percent and more of salt.
- (3) They can grow at the alkaline pH of 9.6.
- (4) They can grow over a wide range of temperatures, some multiplying at as low as 5 to 8°C and most of them at as high as 48 to 50°C.

The term "fecal streptococci" is often used in the food industry to describe those enterococci which are used as indicator organisms.

Genus *Streptomyces* Members of this genus can cause undesirable flavors and appearance when growing on foods; musty or earthy odors and tastes from these organisms may be absorbed by nearby foods when growth of the *Streptomyces* is near at hand.

Genus *Vibrio* Bacteria in this genus are widely distributed in fresh and salt water, in soil, and in the alimentary canal of humans and animals. Some are moderately halophilic. Some species are pathogenic to humans.

Genus *Yersinia* Can be found in the soil. *Y. pestis* is the causative-organism of plague in humans and in rats and other rodents. Some strains of *Y. enterocolitica* are also pathogenic and causative agents of food-borne disease outbreaks.

Groups of Bacteria important in food Bacteriology

1. Lactic acid – forming bacteria or lactics:

These bacteria ferment sugars to lactic acid. This may be desirable in making products such as sauerkraut and cheese. But undesirable in terms of spoilage of wines because they usually form acid rapidly.

Ex: *Leuconostoc*, *Lacto bacillus*, *Streptococcus* and *Pediococcus*.

2. Acetic acid forming bacteria or acetics:

Most of the acetic acid belong to two genera *Acetobacter* and *Gluconobacter*. Both oxidize ethyl alcohol to acetic acid, but *Acetobacter* is capable of oxidizing acetic acid further to CO₂. Characteristics that make acetic acid bacteria important are

1. Their ability to oxidize ethanol to acetic acid.
2. Their strong oxidising power, result in oxidation of desired product like acetic acid, by desirable sps or undesirable sps under favourable conditions.
3. Excessive sliminess of some species

Ex: *Acetobacter acetic sub sp. suboxydans*. This bacteria clog vinegar generators.

3. Butyric acid forming bacteria or butyrics:

Most bacteria of this group are spore forming anaerobes of the genus *Clostridium*.

4. Propionic acid – forming bacteria or propionics: Ex: Propionic bacterium

5. Proteolytic bacteria:

They produce extracellular proteinases proteolytic bacteria may be aerobic, facultative, spore forming, anaerobic and spore forming.

Bacillus cereus – Aerobic, spore forming

Pseudomonas fluorescens – Non spore forming and aerobic to facultative.

Clostridium sporogenes – Spore forming and anaerobic other examples are *Clostridium*, *Bacillus*, *Proteus*. Acid proteolytic bacteria carry on an acid fermentation and proteolysis simultaneously.

Ex: *Streptococcus faecalis var. liquefaciens*

Micrococcus caseolyticus are acid proteolytic.

Some bacteria are putrefactive i.e., they decompose proteins anaerobically to produce foul smelling compounds such as H_2S , mercaptans, amines, indole and fatty acids.

Ex: *Clostridium*, *Proteus*, *Pseudomonas*.

4. Lipolytic Bacteria:

This bacteria produce lipases which catalyze the hydrolysis of fats to fatty acids and glycerol. Many of the aerobic, actively proteolytic bacteria also are lipolytic.

Pseudomonas fluorescens – Strongly lipolytic

Pseudomonas, *Alcaligenes*, *Staphylococcus*, *Serratia* and *Micrococcus* are genera that contain lipolytic bacteria.

5. Saccharolytic bacteria:

These bacteria hydrolyze disaccharides or polysaccharides to simpler sugars. Amylolytic bacteria possess amylase to bring about the hydrolysis of starch outside the cell.

Amylolytic bacteria are *Bacillus subtilis* and *Clostridium butyricum*.

6. Pectinolytic Bacteria:

Pectins are complex carbohydrates that are responsible for cell wall rigidity in vegetables and fruits pectic substances derived from citrus fruits can be used in commercial products as gelling agents.

Ex: *Erwinia*, *Bacillus*, *Clostridium*, *Achromobacter*, *Aeromonas*, *Arthrobacter*, *Flavobacterium*.

7. Thermophilic Bacteria or Thermophiles:

Optimum temperature required for these bacteria 45°C - 55°C. *Bacillus stearothermophilus* – thermophilic flat sour spoilage of low acid canned foods.

8. Thermoduric Bacteria:

Thermoduric bacteria are usually defined as those which can survive a heat treatment such as pasteurization. Ex: *Bacillus* sps, Micrococci, Enterococci can survive pasteurization of liquid eggs. Fungi like *Byssoschlamys fulva*, *Aspergillus* and *Penicillium* are thermoduric. Some thermoduric bacteria like *Bacillus* and enterococci can also be psychrotrophic.

9. Psychrotrophic Bacteria or psychrotrophs:

These bacteria are able to grow at commercial refrigeration temperatures. Unlike psychrophiles, psychrotrophs do not have their optimal temperature for growth at refrigeration temperature and their optimum between 25°C and 30°C.

Ex: *Pseudomonas*, *Flavobacterium*, *Achromobacter* and *Alcaligenes*, *Micrococcus*, *Lactobacillus* etc.

10. Halophilic Bacteria or Halophiles:

Halophilic Bacteria require certain minimal concentrations of dissolved sodium chloride for growth.

Ex: *Pseudomonas*, *Moraxella*, *Acinetobacter*, *Flavobacterium*, *Vibrio* sps which grow best in media with 0.5 – 3.0 percent salt. These micro organisms are isolated from fish shell fish. These are slightly halophilic.

Moderate halophiles are grown in the media.

Containing 3.0 – 15% salt salted fish, brined fish, brined meats and some salted vegetables.

Extreme halophiles grow in the heavily brined foods 15 – 30% salt.

EX: *Halobacterium*, *Halococcus*.

Other bacteria are salt tolerant i.e., halotolerant bacteria can grow with or without salt. Usually they are capable of growing in foods containing 5.0% salt or more. Ex: *Bacillus*, *Micrococcus*, *Corynebacterium*, *Streptococcus* and *Clostridium* sps., *Sarcina*, *pediococcus*, *Alcaligenes*.

11. Osmophilic or Saccharophilic Bacteria:

Osmophilic bacteria are those which grow in high concentrations of sugar. Ex: *Leuconostoc*.

12. Pigmented Bacteria:

Colors produced by pigmented bacteria growing on or in foods.

Flavobacterium – Yellow to orange; *Serratia* – Red; *Halobacterium* – Pink

13. Slime or rope forming bacteria:

Alcaligenes viscolactis, *Enterobacter aerogenes* & *Klebsiella oxytoca* causes ropiness of milk and *Leuconostoc* spp., producing slime in sucrose solutions and slimy surface growth of various bacteria occurring on foods.

Streptococcus & *Lactobacillus* make milk slimy or ropy. *Micrococcus* makes curing solutions for meats ropy. *Lactobacillus plantarum* and *Lactobacilli* may cause ropiness in various fruit, vegetable and grain products e.g. in cider, sauerkraut and beer.

14. Gas forming Bacteria:

Many kinds of bacteria produce small amounts of gas and yield it slowly.

Ex: *Leuconostoc*, *Lactobacillus* (heterofermentative), Propionic bacterium, *Escherichia*, *Enterobacter*, *Proteus*, *Bacillus* and *Clostridium*.

Leuconostoc, *Lactobacillus*, *Propionibacterium*, produces only CO₂.

Other genera produce both CO₂ and H₂.

15. Coliform and Fecal coliform group:

Coliforms are short rods that are defined as aerobic and facultative anaerobic, gram negative, non spore forming bacteria. Ex: *Escherichia coli*, *Enterobacter aerogenes*.

Fecal coliform group includes coliforms capable of growing at 44 - 45°C.

Geotrichum candidum is the machinery mold and as an indicator of plant sanitation and contaminated equipment. Some of the characteristics that make the coliform bacteria important in food spoilage are

1. Their ability to grow well in a variety of substrates and synthesise most of the necessary vitamins.
2. Their ability of the group to grow well over a fairly wide range of temperatures from below 10°C to about 46°C.
3. Their ability to produce considerable amounts of acid and gas from sugars.
4. Their ability to cause off – flavours often described as unclean or barny.
5. Their ability of *E. aerogenes* to cause sliminess or ropiness of foods.

LECTURE-8

Principles of Food Preservation. Methods of Food preservation, application in food preservation

Principle of food preservation:

Prevention or delay of microbial decomposition

By keeping out micro organisms (asepsis)

By removal of micro organisms e.g., by filtration.

By hindering the growth and activity of micro organisms e.g., by low temperatures, drying, anaerobic conditions or chemicals.

By killing the micro organisms e.g. by heat or radiation.

Prevention or delay of self decomposition of the food.

By destruction or inactivation of food enzymes e.g., by blanching.

By prevention or delay of purely chemical reactions e.g., prevention of oxidation by means of an antioxidant.

Prevention of damage because of insects, animals, mechanical causes etc.

Principles of Food Preservation:

In accomplishing the preservation of foods by the various methods, the following principles are involved:

1. Prevention or delay of microbial decomposition
 - a. By keeping out microorganisms (asepsis)
 - b. By removal of microorganisms, e.g., by filtration
 - c. By hindering the growth and activity of microorganisms, e.g., by low temperature, drying, anaerobic conditions, or chemicals.
 - d. By killing the microorganisms, e.g., by heat or radiation
2. Prevention or delay of self-decomposition of the food.
 - a. By destruction or inactivation of food enzymes, e.g., by blanching
 - b. By prevention or delay of purely chemical reactions, e.g., prevention of oxidation by means of an antioxidant
3. Prevention of damage because of insects, animals, mechanical caused, etc., a subject beyond the scope of this text

The methods used to control the activities of microorganisms usually are effective against enzymatic activity in the food or chemical reactions. Methods such as drying and the use of low temperatures, however, permit auto decomposition to continue unless special precautions

are taken. For example, most vegetables are blanched (heated) to inactivate their enzymes before being frozen.

Applications to Food Preservation:

Especially important in food preservation (i.e., prevention of spoilage) is the lengthening, as much as possible, of the lag phase and the phase of positive acceleration. This can be accomplished in different ways:

1. By introducing as few spoilage organisms as possible, i.e., by reducing the amount of contamination; the fewer organisms present, the longer the lag phase.
2. By avoiding the addition of actively growing organisms (from the logarithmic phase of growth). Such organisms might be growing on unclean containers, equipment, or utensils that come in contact with foods.
3. By one or more unfavourable environmental conditions: unfavourable food, moisture, temperature, pH, or O-R potential, or presence of inhibitors. The more unfavourable the conditions, the longer the delay of the initiation of growth.
4. By actual damage to organisms by processing methods such as heating or irradiation. Thus, for example, bacteria or their spores subjected to sublethal heat treatments have been found to require a better culture medium for growth than do the unheated organisms. Often a combination of methods for delaying the initiation of growth is enough to give a food the desired storage life.

LECTURE-9

Asepsis, removal of Micro organisms. Maintenance of Anaerobic conditions. General principles of food preservation

Asepsis:

It is the keeping out micro organisms as a preservative factor. The inner tissues of healthy plants and animals usually are free from micro organisms. If there is a protective covering about the food, microbial decomposition is delayed or prevented.

Ex: Shells of nuts, skins of fruits & vegetables, Husks of ear corn, shells of egg. Fat on meat or fish.

If protection covering has been damaged, decomposition spread from the outer surface that the inner tissues are subject to decomposition by micro organisms.

In food industries an increasing amount of attention is being given to the prevention of the contamination of foods, from the raw material to the finished product.

Packaging of foods is a widely used application of asepsis. The covering may range from to hermetically sealed container contamination during handling.

In dairy industry, contamination with micro organisms is avoided from the production to handling of milk in the market. Quality of the milk is judged by its bacterial content. In the canning industry, bio burden of micro organisms determines the heat process necessary for the preservation of food.

In the meat – packing industry sanitary methods of slaughter, handling and processing reduce the load and thus improve the keeping quality of the meat or meat products.

In industries involving controlled food fermentation e.g., in cheese making, the fewer the competing organisms in the fermenting material, the more likely the success of the fermentation.

Removal of Micro organisms:

Removal of micro organisms is not very effective in food preservation but under special conditions it may be helpful. Removal may be accomplished by means of filtration, centrifugation, washing, trimming.

a) Filtration:

It is the only successful method for the complete removal of organisms and its use is limited to clear liquids. The liquid is filtered through a previously sterilized “bacterial proof” filter made of sintered glass, diatomaceous earth, material and the liquid is forced through by positive or negative pressure. This method has been used successfully with fruit juices, beer, soft drinks, wine and water.

b) Centrifugation or Sedimentation:

It is not very effective. All micro organisms are not removed.

Ex: 1) Used in the treatment of drinking water but it is insufficient to remove micro organisms

2) In the milk, main purpose of centrifugation is not to remove bacteria but to take out other suspended materials.

c) Washing:

Washing raw foods remove spoilage micro organisms.

Ex: Cabbage heads or cucumbers before their fermentation into sauerkraut and pickles, removes most of the soil micro organisms on the surface.

Washing fresh fruits and vegetables removes soil organisms that may be resistant to the heat process during canning.

Washing foods is dangerous if water adds spoilage micro organisms and increases the moisture so that growth of spoilage organisms is encouraged.

d) Trimming:

Spoiled portions of a food removed by trimming. Large no. of spoilage organisms are removed by this way.

Ex: Trimming the outer leaves of cabbage heads is recommended for the manufacture of sauerkraut.

Maintenance of Anaerobic conditions:

Scaled, packaged foods may have the anaerobic conditions in the container.

A complete fill, evacuation of the unfilled space (the head space in a can) or replacement of the air by CO₂ or by an inert gas such as nitrogen will bring about anaerobic conditions.

Spores of aerobic bacteria may resistant to heat and survive in canned foods but unable to grow in the absence of O₂.

LECTURE-10

Food Preservation by use of high temperature. Factors affecting heat resistance (Thermal death time). Heat resistance of Microorganisms and their spores.

Preservation by use of High temperatures

Killing of micro organisms by heat is supposed to be caused by the denaturation of the proteins and by the inactivation of enzymes required for metabolism.

Factors affecting heat resistance (Thermal death time):

Cells and spores of microorganisms differ widely in their resistance to high temperatures.

A → B – small number of cells have low resistance

B → C – most of the cells have a medium resistance

C → D – a small number have high resistance points

Certain factors are known to affect the heat resistance of cells or spores. Chief known factors are as follows.

1. The temperature – time relationship: The time for killing cells or spores under a given set of conditions decreases as the temperature is increased.

2. Initial concentration of spores (or cells): The more spores or cells present, the greater the heat treatment necessary to kill all of them.

3. Previous history of the vegetative cells or spores: The conditions under which the cells have been grown and spores have been produced and their treatment thereafter will influence their resistance to heat.

a) Culture medium: If medium is better for growth, the more resistant the cells or spores. The presence of an adequate supply of accessory growth factors usually favors the production of heat resistant cells or spores. Vegetable infusions and liver extracts increase heat resistance. Spores formed and aged in soil or oats are more resistant than those formed in both or agar.

Glucose in a medium increases resistance, where as sugar may result in the formation of enough acid to cause decreased heat resistance.

Phosphate and magnesium ions decrease the heat resistance of bacterial spores produced in a medium containing them. Prolonged exposure to metabolic products reduces the heat resistance of cells & spores.

b) Temperature of incubation: Resistance increases as the incubation temperature is raised toward the optimum for the organism and for many organisms increases further as the temperature approaches the maximum for growth.

E. coli when grown at 38.5°C is more heat resistant than at 28°C.

c) Phases of growth or age: Bacterial cells know their greatest resistance during the late lag phase but almost as great resistance during their maximum stationary phase, followed by a decline in resistance.

Very young spores are less resistant than mature ones. Some spores increase in resistance during the first weeks of storage but later begin to decrease in resistance.

d) Dessication: Dried spores of some bacteria are harder to kill by heat than are those kept moist, but this is not for all bacterial spores.

4. Composition of the substrate in which cells or spores are heated.

a) Moisture: Moist heat is a much more effective killing agent than dry heat. Dry materials require more heat for sterilization than moist ones.

b) Hydrogen – ion concentration (pH) : Cells or spores are most heat resistant in a substrate that is at or near neutrality. Change toward the acid side is more effective than a corresponding increase in alkalinity.

Cameron divided canned foods into:

Acid food – $P^H < 4.5$

Low acid foods – $P^H > 4.5$

Acid foods – Fruit & Certain vegetable products

Low acid foods – Meat, seafood, milk and most of the common vegetables.

Cameron suggested further subdivision of foods based on pH:

1) Low acid foods: pH above 5.3

Ex: Peas, corn, lima beans, meats, fish, poultry and milk

2) Medium acid foods: pH between 5.3 and 4.5

Ex: Spinach, Asparagus, Beets and pumpkin.

3) Acid foods: pH between 4.5 – 3.7

Ex: Tomatoes, pears and pine apple.

4) High acid foods: pH 3.7 and below

Ex: Berries and sauerkraut

Heating of food at high temperatures causes a decrease in the pH of low or medium acid foods.

C) Other constituents of the substrate:

The salt present in appreciable amounts in most foods is sodium chloride. NaCl have protective effect on some spores.

Sugar protects some organisms or spores but not others. It is high for some osmophilic organisms and low for others, high for spores and low for non osmophilic cells. Sugar decreases a_w (water activity). Reduced a_w does result in an increase in heat resistance.

Solutes like glucose protects *Escherichia coli* and *pseudomonas fluorescens* against heat better than sodium chloride at a_w levels near the minimum for growth.

Glucose have no protective effect on *Staphylococcus aureus*, where as NaCl is very protective.

High soluble solids foods – syrups and concentrates

Low soluble solids foods – Fruits, vegetables and meats.

Colloidal materials, especially proteins and fats are protective against heat.

Antiseptic or germicidal substances in the substrate help heat in the destruction of organisms. Thus H_2O_2 and heat is used to reduce the bacterial content of sugar and is the basis of a process for milk.

Heat Resistance of Micro organisms and their spores:

Heat resistance of micro organisms usually is expressed in terms of their thermal death time. (TDT) and TDT defined as the time it number of organisms under specified conditions. TDT also referred to as the absolute thermal death time to distinguish it from the majority thermal death time for killing most of the cells or spores present and the thermal death rate, expressed as the rate of killing.

Thermal death point (TDP) is the temperature necessary to kill all the organisms in 10 minutes.

Heat resistance of yeasts and yeast spores:

Resistance of yeasts and their spores to moist heat varies with the species and even the strain with the substrate in which they are heated.

Ascospores of yeasts need 5-10°C more heat than vegetative cells.

Most ascospores are killed at 60°C for 10-15 min vegetative yeasts usually are killed by 50-58°C for 10-15 min. both yeasts and their spores are killed by the pasteurization temperatures.

Heat Resistance of Molds and mold spores:

Asexual spores are more resistant than ordinary mycelium.

Aspergillus, *Penicillium* and *Mucor* are more resistant to heat than other molds.

Byssoschlamys fulva – Very heat resistance mold on sclerotia are difficult to kill by heat. Some can survive a heat treatment of 90 - 100°C. Mold spores are fairly resistant to dry heat.

Heat resistance of bacteria and bacterial spores:

Cocci usually are more resistant than rods although there are many exceptions.

The higher the optimal and maximal temperatures for growth, the greater the resistance to heat is likely to be.

The bacteria that clump considerably or form capsules are more difficult to kill than those which do not form capsules.

The cells high in lipid content are harder to kill than other cells.

Heat resistance of Enzymes:

Although most food and microbial enzymes are destroyed at 79.4°C, some may withstand higher temperatures, especially if high temperature short time heating is employed.

Thermal processes designed to inactivate micro organisms will also inactivate enzymes of concern.

Some hydrolases (proteinases and lipases) will retain a substantial level of activity after an ultra high temperature process. The residual activity of these enzymes may spoil the processed product during long term storage.

Detection of the bovine phosphatase enzyme in processed milk usually indicates that the milk was not properly pasteurized.

Heat penetration:

The rate of penetration of heat into food must be known in order to calculate the thermal process necessary for its preservation. Since every part of the food in a can or other container must receive an adequate heat treatment to prevent spoilage.

Heat penetration from an external source to the center of the can may take place by conduction, where heat passes from molecule to molecule, by convection where heat is transferred by movement of liquids or gases or some times by combination of conduction and convection conduction is slow in foods and rapid in metals.

When both conduction and convection are involved in the heating of foods, they may function simultaneously or successively. When solid particles of food are suspended in a liquid, the particles heat by conduction and the liquid heats by convection. Some foods change in consistency during heating, and a broken heating curve results.

Ex: Sugar syrups, Brine packed whole grain corn, certain thick soups and tomato juices.

Factors that determine the time required to bring the center of the container of food up to the sterilizing temperature are as follows:

1. The material of which the container is made. Glass has a slower rate of heat penetration than a metal can.

2. The size and shape of the container. The larger a can is, the longer it will take to reach a given temperature at the center because the distance to the center of the larger can is greater and it has less surface per volume or weight. Hence larger cans are heated longer proportionally but not to as high a temperature at the center.

3. Initial temperature of the food.

The temperature of the food in a can when it goes into the retort (steam sterilizer) makes no difference in time required for the center of the can to reach the retort temperature.

A high initial temperature is important in processing canned foods that heat slowly, such as cream style corn, pumpkin and meat.

4. Retort temperature:

Food cans placed in steam sterilizer of different temperatures reach the respective temperatures at the same time.

5. Consistency of can contents and size and shape of pieces:

All of these are important in their effect on heat penetration. Pieces that retain their identity i.e., do not cook apart. Ex: Peas, plums, beets, Asparagus whole grain corn. If the pieces are large, heating is delayed, because the heat must penetrate to the center of the pieces before the liquor can reach the retort temperature. Large beets or large stalks of Asparagus heat more slowly than small ones.

b) Pieces that cook apart and become mushy or viscous:

These heat slowly, because heat penetration becomes mostly by conduction rather than convection.

Ex: Cream style corn, squash, pumpkin and sweet potatoes.

c) Pieces that layer:

Asparagus layers vertically, hence, convection currents travel mostly up and down. Spinach layers horizontally, producing a “baffle board affect” which interferes with convection currents. Layering is greatly affected by the degree of fill of the can.

Consistency of the can contents is affected by some of the sauces added.

Tomato sauce on baked beans slows down heat penetration more than plain sauce does.

Rate of heat penetration decreases with increasing concentrations of sugar.

6. Rotation and agitation:

Rotation or agitation of the container of food during heat processing will hasten heat penetration if the food is fluid, but it may also cause physical changes in foods.

Rotation is used successfully with canned evaporated milk, and shaking is used in foods in the form of pastes or purees.

Brined, whole kernel corn employs heating in a continuous cooker of high boiling liquid with the can contents mixed by being on the periphery of a rotating reel or by rotation on rollers.

LECTURE-11

Determination of heat resistance. Heat penetration – Pasteurization, Heating at about 100°C. Heating above 100°C, canning. [Note: Determination of Thermal processes, Methods TDT curves will be dealt in practicals]

Determination of thermal process: Determination of thermal process by

1. TDT curves
2. Heat penetration and cooling curves
3. Three methods are used to determine the heat process
4. Graphical method
5. Formula method
6. Nomo gram method

By each of these methods equivalent processes can be calculated.

Heat treatments employed in processing foods:

The various degrees of heating used on foods might be classified as

1. Pasteurization
2. Heating at about 100°C
3. Heating above 100°C

1. Pasteurization:

Pasteurization is a heat treatment that kills part but not all of the microorganisms present in foods and the temperature applied is below 100°C. Heating may be by steam, hot water, dry heat or electric currents. Products are cooled promptly after the heat treatment.

Pasteurization is used

1. When more rigorous heat treatments might harm the quality of the product.
2. To kill pathogens in milk.
3. When the main spoilage organisms are not very heat resistant (Ex Yeasts in fruit juices).
4. When competing microorganisms are to be killed, allowing a desired fermentation

Preservative methods used to supplement pasteurization include

Refrigeration Ex: Milk

Keeping out micro organisms Ex: Packaging

Maintenance of anaerobic conditions Ex: Sealed containers

Addition of high concentrations of sugar. Ex: Sweetened condensed milk

Presence or addition of chemical preservatives. Ex: Organic acids on pickles.

Methods of pasteurization:

HTST Method: (High temperature short time)

In this method, high temperature is employed for a short time. Temperature is 71.7°C and time is 15 sec.

LTLT Method: (Low temperature long time)

In this method, low temperature is employed for a longer time.

Temperature is 62.8°C and time is 30 minutes time. Ex: Milk

Ultra pasteurization: In this method, the temperature applied is 137.8°C for at least 2 seconds.

Earlier pasteurization temperature was set based on pathogenic organism present in milk *Mycobacterium tuberculosis*. This bacterium killed at 61.7°C, but other organism *Coxiella burnetti*, a rickettsia causing Q fever is survived pasteurization temperature. Hence to kill *Coxiella burnetti*, Pasteurization temperature was raised to 62.8°C. *Coxiella burnetti* is transmitted by milk.

Ice cream mix	71.1°C for 30 min 82.2°C for 16-20 sec
Grape wines	82-85°C for 1minute
Fruit wines	62.8°C
Beer	60°C
Dried fruits	65.6-85°C for 30-90 min

Heating at about 100°C:

This treatment was sufficient to kill everything but bacterial spores in the food and it is sufficient to preserve even low and medium acid foods.

Many acid foods can be processed successfully at 100°C or less. Ex: sauerkraut & highly acid fruits. 100°C temperature is obtained by boiling a liquid food or by immersion of the container of food in boiling water or by exposure to following steam.

Very acid foods Ex: Sauerkraut may be preheated to a temperature below 100°C, packaged hot and not further heat processed. Blanching fresh vegetables before freezing or drying involves heating briefly at about 100°C.

a) Baking: Internal temperature of bread, cake or other bakery products approaches but never reaches 100°C as long as moisture is present. The temperature of unsealed canned containers heated in the oven cannot exceed the boiling temperature of the liquid present. Bacterial spores that survive baking temperature may cause ropiness.

b) Simmering: It is the incipient or gentle boiling with the temperature about 100°C.

c) Roasting: Ex: Meat. In meat internal temperature reaches only about 60°C in rare beef, up to 80°C in well done beef, 85°C in a pork roast.

d) Frying: In frying, outside of the food is very hot, but the center ordinarily does not reach 100°C.

e) Cooking: Cooking is an indefinite term with little meaning. However the term “Cook” implies a specific time and temperature for a thermal process.

f) Warming up: Warming up a food may mean anything from a small increase in temperature up to heating to 100°C.

Heating above 100°C:

Temperatures above 100°C usually are obtained by means of steam under pressure in steam pressure sterilizers or retorts. The temperature in the retorts increases with rising steam pressures.

Ex: Milk can be heated to temperatures up to 150°C by use of steam injection or steam infusion followed by flash evaporation of the condensed steam and rapid cooling. Processes such as this for milk have been referred to as ultrahigh temperature or UHT processes.

Canning:

Canning is defined as the preservation of foods in sealed containers and usually implies heat treatment to prevent spoilage.

Canning is done in “tin cans” made of tin coated steel or in glass containers, aluminium, plastics as pouches or solid containers, aluminium, plastics as pouches or solid containers or of a composite of materials. Therefore the word “canning” is a general term and often replaced by “hermetically sealed containers”.

Spallanzani (1765) preserved food by heating it in a sealed container.

Nicolas Appert called “Father of canning” because he did experiments on the heating of foods in sealed containers and given directions for preservation by canning.

Appertization:

Preservation of foods by canning with the application of heat treatments in cork stoppered, wide mouthed glass bottles.

Around 1850, European workers began using baths of oil, salt brine or calcium chloride solution to obtain temperatures above 100°C. 1860 – Solomon introduced the use of CaCl_2 and reducing the process time from 5 to 6 hrs to half an hour and helping the canning in American civil war.

Appert original container – cork stoppered, wide mouthed glass jar.

‘Tin canister’ abbreviated by Americans to “tin can” was patented by Peter Durand in England in 1810.

Most modern cans are made of steel plate coated with tin. Enamels are coated onto flat sheets of plate before the manufacture of cans to prevent or slow discoloration or corrosion.

Sanitary or standard, enamel is used for cans for highly colored fruits and berries or for beets to prevent the fading of color caused by tin plate.

C – Enamel contains zincoxide, so that the white Zn S is formed instead of dark Fe S when low acid, Sulfur bearing foods such as corn are canned, and dark erring of the interior of the can is thus avoided.

This enamel is not usable for meat, because fat would loosen and flake the enamel.

Special enamels are employed for certain foods Ex: Milk, meats, wine and beer, soups.

Glass containers: are used for the canning of many foods and have been greatly improved since the days of Appert.

Aluminium containers are available but do not withstand strong mechanical stresses. Hence they are used for products that do not require high vacuums or high temperature processing e.g., beer, frozen fruits, frozen juice concentrates and cheese.

Flexible pouches or bags made of plastic or of plastic laminated with foil are used for packaging frozen dried or unprocessed foods.

Pressurized Packaged Foods:

Pressurized packaging liquids or pastes called aerosols, are packed under pressure of a propellant gas, usually CO₂, N₂ or nitrous oxide, so as to dispense the food as a foam, spray or liquid. Many foods are now being so packaged, e.g., whipped cream and other toppings, beverage concentrates, salad dressings, condiments, oils, jellies and flavouring substances.

Acid foods may be heated, canned and then gassed but the gassing process may contaminate the food. Aseptic canning is a possibility for low and medium acid foods.

Nitrogen would inhibit aerobes.

CO₂ would inhibit aerobes. The both are known as obligate aerobes. CO₂ does not inhibit lactic acid bacteria, *Bacillus coagulans*, *Streptococcus faecalis* or yeasts.

Nitrous oxide represses oxide represses some fungi.

Canning in the Home:

Home canners have used processes a similar to those of Appert with the temperature of heating not exceeding 100°C.

TDP (Thermal Death Point):

Temperature required to kill known no. of micro organisms at a given time.

Here temperature is unknown.

TDT (Thermal Death Time):

Time required to kill known no. of micro organisms at a given temperature.

DRT (Decimal Reduction Time):

Time required to reduce the microbial population at specified temperature. It is designated as “D”

Book published by N. Appert.

“The book for all households; or the art of preserving animal and vegetable substances for many years”.

LECTURE-12

Preservation by use of low temperatures. Growth microorganisms at low temperatures. Common or Cellar storage. Chilling or cold storage. Freezing or Frozen storage. Sharp Freezing and quick freezing. Changes during freezing, storage and thawing

Preservation by use of Low temperatures

Low temperatures are used to retard chemical reactions and action of food enzymes and to slow down or stop the growth and activity of micro organisms in food.

Growth of Micro organisms at low temperatures:

Freezing prevents the growth of most food borne micro organisms and refrigeration temperature slow growth rates.

Clostridium botulinum type E has a minimum temperature for growth of about 3.3°C.

Yersinia enterocolitica grow at temperatures 0-3°C.

Molds like *Cladosporium* and *Sporotrichum*, *Penicillium* and *Monilia* at -4°C.

Temperatures employed in low temperature storage:

1) Common or cellar storage:

The temperature in common or cellar storage usually lower than 15°C.

Root crops, potatoes, cabbage, celery, apples stored for limited periods. The deterioration of such fruits and vegetables by their own enzymes and by micro organisms is not prevented but is slower than at atmospheric temperatures. Low humidity in the storage cellar results in losses of moisture from the stored food and too high humidity favours spoilage by micro organisms.

Chilling or cold storage:

Chilling storage is at temperatures not far above freezing and usually involves cooling by ice or mechanical refrigeration.

Eggs, dairy products, meats, seafood, vegetables and fruits may be held in chilling storage for a limited time.

Enzymatic and microbial changes in the foods are not prevented but are slowed considerably. Chilling temperature is selected on the basis of the kind of food and the time and conditions of storage.

Ex: Banana stored and keeps best at 13.3 – 16.7°C

Sweet potatoes keep best at 10-12.8°C.

Relative humidity in the chilling storage varies with the food stored and with environmental factors such as temperature, composition of the atmosphere and ray treatments.

Changes in humidity as well as in temperature during storage may cause “sweating” or precipitation of moisture on the food. A moist surface favours microbial spoilage.

Ventilation or control of air velocities of the storage room is important in maintaining a uniform relative humidity throughout the room, removing odors and preventing the development of stable odors and flavours.

The amounts and proportions of gases in the storage atmosphere influence preservation by chilling. Stored plant foods continue to respire, using oxygen and giving off carbon dioxide.

In “Gas storage” of foods, where the composition of the atmosphere has been controlled by the introduction of CO₂, Ozone or other gas or the removal of CO₂.

Gas storage ordinarily is combined with chilling storage. In the presence of optimal concentrations of carbon dioxide or ozone the following advantages are present

A food will remain unspoiled for a longer period. Higher relative humidity can be maintained without harm to the keeping quality of certain foods.

Higher storage temperature can be used without shortening the keeping time of the food.

2.5% CO₂ to be best for eggs

10.0% CO₂ for chilled beef

100% CO₂ for Bacon.

Combination of U.V. irradiation with chilling storage helps preserve some foods and may permit the use of a higher humidity or storage temperature than with chilling alone. U.V. lamps have been installed in rooms for the storage of meat and cheese.

Freezing or frozen storage:

The storage of foods in the frozen condition has been an important preservative method for centuries. Under frozen storage, microbial growth is prevented entirely and the action of food enzymes is greatly retarded. The lower the storage temperature, the slower will be any chemical or enzymatic reactions.

Fruits and vegetables are selected on the basis of their suitability for freezing and their maturity and are washed, trimmed, cut or otherwise pretreated as desired.

Vegetables are scalded or blanched. Fruits are packed in syrup.

Most foods are packaged before freezing but strawberries are frozen before packaging.

Scalding or blanching of vegetables is done with hot water or steam and has the following advantages

1. Inactivation of most of the plant enzymes which cause toughness, change in colour, mustiness, loss in flavour, softening and loss in nutritive value.

2. Reduction in the no. of micro organisms on the food.
3. Enhancement of the green color of vegetables such as spinach.
4. Displacement of air entrapped in the tissues.

Types of freezing:

1. Sharp freezing or slow freezing; 2. Quick freezing; 3. Dehydro freezing

Sharp freezing	Quick freezing
1. It refers to freezing in air with only natural air circulation or with electric fans. 2. Temperature usually -23.3°C or lower but may vary from -15 to -29°C. 3. Time required to achieve sharp freezing is 3-72 hrs. 4. Large ice crystals are formed. More mechanical damage of food is observed. 5. Longer period of solidification.	1. It refers to freezing foods in relatively short time (30 min). 2. Temperature usually between -17.8 to 45.6°C if it is indirect contact with refrigerant. (or) -17.8°C to -34.4°C if it is done with air blast freezing. 3. Time required to achieve above temperatures is 30 min or lower. 4. Small ice crystals are formed hence there is less mechanical damage of food. 5. Shorter period of solidification and therefore less time for diffusion of soluble materials and separation of ice.

Advantages of Quick Freezing over slow freezing:

1. Smaller ice crystals are formed; hence there is less mechanical destruction of intact cells of the food.
2. There is a shorter period of solidification of ice
3. There is more prompt prevention of microbial growth
4. There is more rapid slowing of enzyme action.
5. Quick frozen foods are supposed to bring to the room temperature before cooking or consumption. This process is called thawing. Ex: Vegetables, meat.

Dehydrofreezing:

Fruits and vegetables have about half their moisture removed before freezing.

Certain foods like fruits, vegetables, fish, shrimp and mushrooms now are being frozen by means of liquid nitrogen.

Changes during preparation for Freezing:

Rate and kind of deterioration of foods before freezing will depend on the condition of the food at harvesting or slaughter and the methods of handling. The temperatures at which the food is held and other environmental conditions will determine the kinds of micro organisms to grow and the changes to be produced.

The condition of the food at the time of freezing will determine the potential quality of the frozen food.

Changes during Freezing:

Quick freezing process rapidly slows chemical and enzymatic reactions in the foods and stops microbial growth. A similar effect is produced by sharp or slow freezing but with less rapidity.

Physical effects like expansion in volume of the frozen food and ice crystals form and grow in size. Ice crystals form and grow in size.

Ice crystals are usually larger with slow freezing and more ice accumulates between tissues cells than with quick freezing and may crush cells.

Water is drawn from the cells to form such ice and results in increase in the concentration of solutes in the unfrozen liquor and leads to salting out dehydration, de-naturation of proteins and causes irreversible changes in colloidal systems such as the syneresis of hydrophilic colloids.

Changes during storage:

During storage of the food in the frozen condition chemical and enzymatic reactions proceed slowly. Meat, poultry and fish proteins may become irreversibly dehydrated, the red myoglobin of meat may be oxidized – especially at surfaces to brown met myoglobin. Fats of meat and fish may become oxidized and hydrolyzed. The unfrozen, concentrated solution of sugars, salts etc may ooze from packaging of fruits or concentrates during storage as a viscous material called metacryotic liquid. Fluctuation in the storage temperature results in growth in the size of ice crystals and in physical damage to the food.

Dessication of the food is likely to take place at its surface during storage. When ice crystals evaporate from an area at the surface a defect called freezer burn is produced on fruits, vegetables, meat, poultry and fish. The spot usually appears dry, grainy and brownish and the tissues become dry and tough. At freezing temperatures vegetative cells of micro organisms that are unable to multiply will die.

Changes during thawing:

When the ice crystal melts, the liquid either is absorbed back into the tissue cells or leaks out from the food. Slow, well controlled thawing usually results in better return of moisture to the cells than rapid thawing.

The pink or reddish liquid that comes from meat on thawing is called drip or bleeding. The liquid oozing from fruits or vegetables on thawing is termed leakage.

The wilting or flabbiness of vegetables and the mushiness of fruits on thawing are chiefly due to physical damage during freezing.

During thawing, enzymatic action increases. Kinds of micro organisms growing depend on the temperatures of thawing and the time the food was allowed to stand after thawing.

Effect of subfreezing and freezing temperature on micro organisms:

a) Lethal effects: Many cells are killed by freezing but this is not a sterilization procedure. One of the most widely used techniques for the preservation of cultures is by freezing and frozen storage, usually in liquid nitrogen. Lethal effects are thought to be the result of de-naturation or flocculation of essential cell proteins or enzymes.

Rapid cooling of cells from an optimal temperature to 0°C can also result in death. This observation is referred to as **cold shock** and it is related to alterations of lipids in the membrane by damaging permeability of cell.

b) Sublethal effects: Microbial cells that are damaged or injured during frozen state, which will not give good counts during enumeration of frozen foods. Cells in this state have been referred to as **freeze injured**, **frost injured** or **metabolically injured**. Freezing of micro organisms in a food may therefore result in “**cryo injury**”. These cells can be recovered if repair time is permitted or additional nutritional factors are added to the enumeration media.

c) Response of micro organisms to Freezing:

1) The kind of micro organisms and its state: Resistance to freezing varies with the kind of micro organism, its phase of growth and whether it is a vegetative cell or a spore.

Micro organisms based on sensitivity to freezing classified as a) susceptible b) moderately resistant c) insensitive organisms.

a) Susceptible: Vegetative cells of yeast and molds and many Gr –ve bacteria.

b) Moderately resistant: Gram +ve organisms including *Staphylococci* and *Enterococci*.

c) Insensitive organisms: Sporeformers.

2. Freezing state: Faster freezing rates would tend to be less destructive since the critical range would be passed through faster.

- 3. The freezing temperature:** High freezing temperature is more lethal or harmful. More organisms are inactivated at -4 to -10°C than at -15 to -30°C.
- 4. The time of frozen storage:** The initial killing rate during freezing is rapid, but it is followed by a gradual reduction of micro organisms and is referred to as storage death. The number of viable organisms decreases with lengthened time of storage.
- 5. The kind of food:** Sugar, salt, proteins, colloids, fat and other substances may be protective, where as high moisture and low P^H may hasten killing.
- 6. Influence of defrosting:** The response of micro organisms to the rate of defrosting varies. Rapid warming has been found to be harmful to some bacteria.
- 7. Alternative freezing and thawing:** Altering freezing and thawing hasten the killing of micro organisms but apparently does not always do so.
- 8. Possible events during freezing of the cell:** As the temperature is lowered more and more water freezes. The remaining or unfrozen free water at each temperature therefore becomes more and more concentrated with solutes (Salts, proteins, nucleic acids etc). This can change the pH of cellulose matter, concentrate electrolytes, alter colloidal states, denature proteins and increase viscosity. Ice crystals can form outside the cell with a resulting dehydration or concentration effect. Intracellular crystals may form and grow or crystallize right through the cell, resulting in altered permeability or holes in the membrane and cell wall. Intracellular ice is thought to be more harmful to cells than are extra cellular ice crystals.

LECTURE-13

Preservation by drying, methods of drying. Treatments of foods before drying. Procedure after drying. Microbiology of dried foods. Intermediate moisture foods

Preservation by Drying

Preservation of foods by drying has been practiced for centuries.

Drying usually is accomplished by the removal of water, but any method that reduces the amount of available moisture i.e., lowers the a_w in a food is a form of drying.

Ex: 1. Dried fish heavily salted to remove moisture.

Grains after harvest dried to minimum moisture levels. Sugar added in sweetened condensed milk to reduce the amount of available moisture.

Sun dried food:

Moisture removed by exposure to the sun's rays without any artificially produced heat and without controlled temperatures, R.H.s air velocities.

Dehydrated or dessicated food:

Dehydrated or desiccated food has been dried by artificially produced heat under controlled conditions of temperature, R.H and air flow. Condensed usually implies that moisture has been removed from a liquid food. Evaporated may have a similar meaning or may be used synonymously with the term dehydrated.

Methods of Drying:

Solar Drying: Solar drying is limited to climates with a hot sun and a dry atmosphere certain fruits such as raisins, prunes, figs, apricots, nectarines, pears and peaches are dried by solar. Fish, rice and other grains are also dried.

Drying by mechanical dryers: Artificial drying involves the passage of heated air with controlled relative humidity over the food to be dried. The simplest dryer is the evaporator or kiln, sometimes used in the farm home.

Forced – draft drying systems employ currents of heated air that move across the food usually in tunnels. Alternative method is moving the food on conveyor belt or on trays in carts through the heated air. Liquid foods like milk, juices and soups may be evaporated by the use of low temperature and vacuum.

Drum drying is the passage of food over a heated drum with or without vacuum. Spray drying is spraying the liquid into a current of dry, heated air.

Freeze Drying:

Certain foods are dried by smoking. Smoking of foods usually has two purposes

Adding desired flavours and has preservation effect. Ex: Meat.

Other methods:

Electronic heating – Removal of still more moisture from food already fairly well dried.

Foam mat drying – liquid food is whipped to foam dried with warm air and crushed to a powder.

Pressure gun puffing – Partially dried foods to give a porous structure that facilitates further drying.

Tower drying – Drying by de humidified air at 30°C or lower used for tomato concentrate, milk and potatoes.

Factors in the control of Drying:

1. The temperature employed
2. The relative humidity of the air.
3. Velocity of the air
4. Time of drying.

Improper control of the factors may cause case hardening resulting from more rapid evaporation of moisture from the surface than diffusion from the interior. It results in hard, horny, impenetrable surface film that hinders further drying.

Treatments of foods before drying:

1. Selection and sorting for size, maturity and soundness
2. Washing especially fruits and vegetables
3. Peeling of fruits and vegetables by hand, machine, lye bath or abrasing
4. Subdivision into halves, slices, shreds or cubes
5. Alkali dipping such as raisins, grapes and prunes. It employs hot 0.1 to 1.5 % lye or sodium carbonate.
6. Blanching or scalding of vegetables and some fruits (apricots, peaches)
7. Sulfuring of light coloured fruits and certain vegetables.

Fruits are sulphured by exposure to SO₂ gas produced by the burning of sulphur 1000 – 3000 ppm depending on the fruit. Vegetables may be sulphured after blanching in a similar manner or by dipping into or spraying with sulfite solution. Sulfuring helps maintain an attractive light. Color, conserve vitamin C and vitamin A and repel insects. It also kills many of the micro organisms present.

Procedures after Drying:

1. **Sweating:** Sweating is storage, usually in bins or boxes for equalization of moisture or readdition of moisture to a desired level. Ex: Almonds, walnuts

2. Packaging: Most foods are packaged soon after drying for protection against moisture, contamination with microorganisms.

3. Pasteurization: Kills pathogens as well as destroying microorganisms.

Microbiology of dried foods:

Before reception at the processing plant:

Fruits and vegetables have soil and water organisms on them when harvested and also contain their own natural surface flora.

Growth of some of these organisms may take place before the foods reach the processing plant. Piled vegetables may heat and support the surface growth of slime forming, flavour – harming, or even rot producing organisms.

Meats and poultry are contaminated by soil, intestinal contents, handlers and equipment. Fish are contaminated by water and by their own slime and intestinal contents as well as by handlers and equipments.

Eggs are dirty by hen, nests and the handlers. Milk is subject to contamination from the cow and may support the growth of some psychrotrophic bacteria.

2. In the plant before drying:

Growth of micro organisms present on foods may continue in the plant up to time of drying. Equipment and workers may contaminate the food. Grading, selection and sorting of foods like fruits, vegetables, eggs and milk will influence the kinds and number of micro organisms present.

Elimination of spoiled fruits and vegetables or of spoiled parts will reduce numbers of organisms in the product to be dried.

Washing fruits and vegetables removes soil and other adhering materials and serve to remove soil and other adhering materials and serve to remove micro organisms. Water used for washing should not add contamination.

Washing eggs may prove more harmful than helpful unless they are used promptly, for the moisture helps bacteria penetrate the shell. Peeling fruits or vegetables with steam or lye reduce number of micro organisms. Slicing or cutting should not increase numbers of organisms.

Dipping in alkali before sun drying may reduce the microbial population (Ex: Certain fruits). Blanching or scalding vegetables reduces bacterial numbers greatly as much as 99%.

Sulfuring of fruits and vegetables also caused a great reduction in numbers of micro organisms and serve to inhibit growth in the dried product.

During the drying process:

Heat applied during drying process causes a reduction in total numbers of micro organisms but the effectiveness varies with the kinds and numbers of organisms originally present and the drying process employed.

Spores of bacteria, molds and vegetative cells of certain bacteria may survive. Improper conditions during drying may even permit the growth of micro organisms. More microorganisms are killed by freezing than by dehydration during the freeze drying process.

After Drying:

If the drying process and storage conditions are adequate, there will be no growth of micro organisms in the dried food. During storage there is a slow decrease in numbers of organisms, more rapid at first and slower thereafter. The micro organisms that are resistant to drying will survive best, therefore the percentages of such organisms will increase.

Ex: Spores of bacteria and molds, micrococci, microbacteria.

Some times there may be contamination of the dried food during packaging and other handling subsequent to drying.

Sweating of dry fruits to equalize moisture may permit some microbial growth.

Microbial content and the temperature of water used to rehydrate dried foods will affect the keeping quality of the rehydrated product. Bacteria in freeze dried chicken meat are further reduced in numbers by rehydration with water at 50°C. Growth of bacteria in the rehydration meat will occur at favourable temperatures, but there is good shelf at 4°C.

Staphylococcus aureus has been found to survive freeze drying and rehydration at 60°C.

Microbiology of specific Dried foods:

Dried fruits: The numbers of micro organisms on most fresh fruits range from comparatively few to many depending on pretreatments. Most dried fruits may; vary from a few hundred per gram of fruit to thousands. They are mostly on the outer surfaces. Spores of bacteria and molds are likely to be the most numerous.

Dried vegetables:

Range from negligible numbers to millions per gram. If drying trays are improperly loaded, souring of such vegetables as onions and potatoes by lactic acid bacteria with a marked increases in numbers of bacteria may take place during the drying process. Risk is greater with onions because they are not blanched.

Ex: Chiefly bacteria are *Escherichia*, *Enterobacter*, *Bacillus*, *Clostridium*, *Micrococcus*, *Pseudomonas*, *Streptococcus*.

Dried Eggs:

Dried eggs may contain from a few hundred micro organisms, mostly bacteria per gram up to over 100 million, depending on the eggs broken and the methods employed.

A variety of kinds of organisms have been found in dried eggs includes Microcci, Streptococci, Coliforms, Spore formers and molds.

Dried Milk:

No. of micro organisms in dry milk may vary from a few hundred per gram to millions.

Predominant kinds of organisms in dry milk are thermophilic Streptococci, Micrococci and spore formers.

Intermediate moisture Foods:

Numerous commercially prepared foods which contain 20 – 40% moisture and have non refrigerated shelf stability have been refined to as intermediate moisture products.

Ex: Soft candies, jams, jellies, honey, many dried fruits, some bakery items, meat products like pepperoni, country ham, Jerky and some dried fish.

These products are also referred as “reduced – water activity products”.

LECTURE-14

Preservation by food additives–The ideal antimicrobial preservatives. Organic acids and their salts, nitrites and nitrates, sulfur dioxide and sulfites. Ethylene and propylene oxide, sugar and salt

Food Additives

Def: A food additive is a substance or mixture of substances, other than the basic food stuff, which is present in food as a result of any aspect of a production, processing, storage or packaging. The term does not include chance contamination.

(Definition given by W.H.O, 1965).

Chemical preservatives:

The food additives which are specifically added to prevent the deterioration or decomposition of a food have been referred to as chemical preservatives.

Factors that influence the effectiveness of chemical preservatives.

1. Concentration of the chemical.
2. Kind, number, age and previous history of the organism.
3. Temperature.
4. Time.
5. The chemical and physical characteristics of the substrate in which the organism is found.

Ideal Antimicrobial preservative:

1. It should have wide range of antimicrobial activity.
2. It should be non toxic to human beings or animals.
3. It should be economical.
4. It should not have an effect on the flavour, taste or aroma of the original food.
5. It should not be inactivated by the food or any substance in the food.
6. It should not encourage the development of resistant strains.
7. It should kill rather than inhibit micro organisms.

Antimicrobial preservatives added to foods can be grouped as:

1. Those added preservatives not defined as such by law:

Natural organic acids (lactic, malic, citric etc.) and their salts, vinegars (acetic is a natural acid), sodium chloride, sugars, spices and their oils, wood smoke, CO₂ and N₂.

2. Substances generally recognized as safe (GRAS) for addition to foods:

Propionic acid, sodium and calcium propionates, caprylic acid, sorbic acid and potassium, sodium & calcium sorbates, Benzoic acid.

3. Chemicals considered to be food additives:

They can be used only when proved safe for humans or animal
Chemicals proved safe and approved by the food & drug administration.

Food Additives and preservatives organic acids and their salts

Organic acids and their salts:

Lactic, acetic, propionic and citric acids or their salts may be added to foods or developed in foods by fermentation.

Citric acid is used in syrups, drinks, jams and jellies as a substitute for fruit flavours and for preservation.

Lactic and acetic acids are added to brines of various kinds, green olives etc.

Propionates – (sodium or calcium propionate) are used most extensively in the prevention of mold growth and rope development in baked goods and for mold inhibition in many cheese foods and spreads. Also used in butter jams, jellies, figs, apple slices and malt extract.

They are effective against molds, little effect on most yeast and bacteria. Their effectiveness decreases with an increase in P^H . optimum activity at P^H 5-6. they are ideal preservatives for bread and baked goods. Propionic acid is a short chain fatty acid ($CH_3 CH_2 COOH$) affects cell membrane permeability.

Propionic acid is found naturally in Swiss cheese up to 1%.

Benzoates:

Sodium salt of benzoic acid has been used extensively as an antimicrobial agent in foods. It is incorporated into jams, jellies, margarine, carbonated beverages, fruits salads, pickles, relishes, fruit salads, pickles, relishes, fruit juices sodium benzoate is relatively ineffective at P^H values near neutrality and the effectiveness increases with increase in acidity. Opt. P^H for sodium benzoate is 2.5 – 4.0.

Two esters of p – hydroxy benzoic acid, methyl paraben and propyl paraben are also used extensively in foods. Butyl and ethyl esters are also used but limited use. These compounds are similar to benzoic acid in their effectiveness. They are effective at higher P^H because the esterification of the carboxyl group.

Sorbates (sorbic acid as the calcium, sodium or potassium salt) used as a spray, dip or coating on packaging materials. It is widely used in cheeses, cheese products, baked goods, beverages, syrups, fruit juices, jellies, jams, fruit cocktails, dried fruits, pickles.

Sorbic acid and its salts are known to inhibit yeast and molds but are less effective against bacteria. They are effective at low P^H Values. ($P^H < 6.0$)

Acetates:

Monochloro acetic acid, paracetic, dehydroacetic acid and sodium diacetate have been recommended as preservatives but not all are approved by FDA.

Dehydroacetic acid has been used to impregnate wrappers for cheese to inhibit the growth of molds, and as a temporary preservative for squash.

Acetic acid in the form of vinegar is used in mayonnaise, pickles, catsup, pickled sausages and pig's test. Acetic acid is more effective against yeasts and bacteria than molds. Its effectiveness increases with decrease in P^H .

Sodium diacetate has been used in cheese spreads and malt syrups and as treatment for wrappers used on butter.

Nitrites and Nitrates:

Combinations of these various salts have been used in curing solutions and curing mixtures for meats. Nitrites decompose to nitric acid which forms nitrosomyoglobin when reacts with the pigments in meats and thereby forms a stable red colour.

Nitrites can react with secondary and tertiary amines to form nitrosamines, which are known to be carcinogenic. Nitrites are currently added in the form of sodium nitrite potassium nitrite, sodium nitrate and potassium nitrate.

Inhibits *C. botulinum* in meat products.

Sulfur dioxide and sulfites:

Egyptians and Romans burned sulphur to form sulphur dioxide a means of sanitizing wine making equipment and storage vessels.

Sulphur dioxide and sulphites are used in the wine industry to sanitize equipment and to reduce the normal flora of the grape must.

They form sulphurous acid and effectiveness is enhanced at low P^H . Affects microbial cells by reduction of disulfide linkages, formation of carbonyl compounds, reaction with ketone groups and inhibition of respiratory mechanisms.

Fumes of burning sulphur are used to treat most light colored dehydrated fruits. SO_2 also used in syrups, fruit juices and wine making. In addition to antimicrobial action of sulfites, they are also used to prevent enzymatic and non enzymatic changes or discoloration in some foods.

Ethylene and propylene oxide:

These two gases are sterilants. Ethylene oxide kills all micro organisms, propylene oxide although it kills many micro organisms but not effective. They act as strong alkylating agents attacking labile hydrogens.

Primarily used as sterilants for packaging materials, fumigation of warehouses and “cold sterilization” of numerous plastics, chemicals, pharmaceuticals, syringes and hospital supplies. FDA restricts the use of ethylene oxide to spices and other processed natural seasonings except mixtures containing added salt. Propylene oxide is permitted only as a package fumigant for dried prunes or glace fruits and as a fumigant for cocoa, gums, spices and processed nut meats.

Sugar and salt:

These compounds lower the water activity (a_w) and have an adverse effect on micro organisms.

Sodium chloride is used in brines and curing solutions or applied directly to the food. Enough may be added to slow down or prevent the growth of micro organisms.

Salt has been reported to have the following effects

1. It causes high osmotic pressure and hence plasmolysis of cells.
2. It dehydrates foods by drawing out and tying up moisture as it dehydrates microbial cells.
3. It ionizes to yield the chlorine ion.
4. It reduces the solubility of oxygen in the moisture
5. It sensitizes the cell against CO_2 .
6. It interferes with the action of proteolytic enzymes.

Sugar such as glucose or sucrose has ability to make water unavailable to organisms by osmotic effect.

LECTURE-15

Preservation by Food Additives – Alcohol, formaldehyde, wood smoke, spices and other condiments and other additives. Other groupings of chemical agents, antibiotics, developed preservatives.

Alcohol:

Ethanol, a coagulant and denaturizer of cell proteins is most germicidal in concentrations between 70 – 95%. Flavoring extracts e.g., vanilla and lemon extracts are preserved by their content of alcohol.

Beer, ale, wine contain alcohol which retards microbial growth. Methanol is poisonous and should not be added to foods. Propylene glycol has been used as a mold inhibitor and as a spray to kill airborne micro organisms.

Formaldehyde:

Addition of formaldehyde to foods is not permitted, except as a minor constituent of wood smoke. This compound is effective against molds, bacteria, and viruses. It is useful in the treatment of walls, shelves, floors etc to eliminate molds and their spores.

Para formaldehyde can be used to control bacterial and fungal growth in tap holes of maple trees. Formaldehyde probably combines with free amino groups of the proteins of cell protoplasm, injures nuclei and coagulates protein.

Wood smoke:

Smoking of foods usually has two main purposes
Adding desired flavours and Preservation effect

Other desirable effects may result in improvement in the colour of the inside of meat, gloss of the outside and a tenderizing action on meats.

Smoking process helps preservation by impregnating the food near the surface with chemical preservatives from the smoke, by combined action of the heat and these preservatives during smoking and by the drying effects, especially at the surface.

Smoke is obtained from burning wood. Like hardwood such as hickory, corncobs or other materials. Other woods such as apple, oak, maple, beech, birch, walnut and mahogany.

Sawdust is added to the fire to give a heavy smudge.

Smoking temperatures for meat vary from 43 - 71°C and the smoking period lasts from a few hours to several days.

Wood smoke contains a large number of volatile compounds that may have bacteriostatic and bactericidal effect. Formaldehyde is considered the most effective with phenols and cresols.

Other compounds in the smoke are aliphatic acids from formic through caproic, primary and secondary alcohols, ketones and acetaldehyde and other aldehydes; waxes; resins; guaiacol and its methyl and propyl isomers; catechol; methyl catechol, pyrogallol and its methyl ester. These compounds sometimes are grouped under the name pyroligneous acid.

Wood smoke is more effective against vegetative cells than bacterial spores. Residual effects of smoke in the food has been reported to be greater against bacteria than against molds. Concentration of mycostatic materials from wood smoke necessary to prevent mold growth increases with a rise in the humidity of the atmosphere of storage.

Spices and other condiments:

Spices and other condiments do not have any marked bacteriostatic effect in the concentrations used, but help other against in preventing the growth of organisms in food.

Mustard flour and volatile oil of mustard are very effective against *Saccharomyces cerevisiae*.

Cinnamon and cloves containing cinnamic aldehyde & eugenol respectively usually are more bacteriostatic than are other spices.

Volatile oil is more effective against yeasts. To be inhibitory to *Bacillus subtilis* and *Escherichia coli*. Acrolein is the active principle in onions and garlic and butyl thiocyanate in horse radish.

Halogens:

Halogens are added to water for washing foods or equipment for cooling and for addition to some products.

Ex: Washing butter, water for drinking may be chlorinated.

Iodine impregnated wrappers used to lengthen the keeping time of fruits. Iodophors which are combinations of iodine with non-ionic wetting agents and acid, are being used in the sanitization of dairy utensils.

Halogens kill organisms by oxidation, injury to cell membranes or direct combination with cell proteins. Hypochlorites, usually calcium or sodium yield hypochlorous acid, a powerful oxidising agent and are effective germicidal agents. Their activity reduces with the presence of organic matter. Hypochlorites are used in the treatment of water. Hypochlorites incorporated in ice for icing fish in transit and in water for washing the exterior of fruits and vegetables.

Phosphoric acid is used in some soft drinks Ex. Colas H_2O_2 also used as a preservative usually in conjunction with heat. Ex: Pasteurization of milk for cheese involves the addition of H_2O_2 and the use of a comparatively low heating temperature. Excess peroxide is decomposed by

catalase. Thermophiles are destroyed in the processing of sugar by a combination of heat and H_2O_2 .

Boric acid and borates still are used in some countries as preservatives for foods. Powdered boric acid has been dusted onto foods Ex: Meats.

Borax (Sodium tetra borate) has been used to wash vegetables and whole fruits such as oranges.

Other groupings of chemical agents:

Peroxides, Bromine, chlorine, iodine, hypochlorites, chloramines and ozone.

Oxidising agents used in bleaching flour are oxides of nitrogen, chlorine, nitrosyl chloride, nitrogen trichloride and benzoyl peroxide.

Mycostats – Propionates, caprylic acid, acetic acid, dehydroacetic acid, monochloro acetic acid sorbic acid and sorbates and propylene glycol of these propionates and sorbates are used heavily.

Mycostatic chemicals that have been tried in vapour or gaseous form are propylene glycol, CO_2 , methyl bromide and phenol derivatives.

Antifungal antibiotics include griseofulvin, pimarinic, fulvic, actidione, rimocidin and nystatin.

Antimycotics permitted in food packaging material are sodium and calcium propionates, sodium benzoate, sorbic acid and sorbates and methyl and propyl parabens caprylic acid used in cheese wraps.

Biphenyl and sodium o-phenyl phenate plus ammonia have been applied to citrus fruits to reduce fungal spoilage.

Boiler – Water additives:

These should be approved by FDA and used for steam that comes in contact with foods.

Antibiotics:

Aureomycin, terramycin and chloramphenicol inhibit protein synthesis.

Nisin used in cheese to suppress the anaerobes.

Natamycin is effective against yeasts and molds.

Natamycin is used in orange juice, fresh fruits, sausage and cheese.

FDA approved the use of chlortetracycline and oxytetracycline dip for preserving poultry.

7 ppm – Uncooked, dressed fowls.

5 ppm – Fresh fish, shucked scallops, unpeeled shrimp.

Developed preservatives:

Food fermentations may serve either or both of two purposes.

1. To produce new and desired flavours; 2. To help preserve the food.

Ex: Fermented milks, sauerkraut.

The preservatives produced in foods by microbial action are for the most part acids and alcohol. The preservative effect of these substances nearly always is supplemented by one or more additional preservative agents such as low temperature, heat, anaerobic conditions, sodium chloride, sugar or added acid.

Developed acidity plays a part in the preservation of sauerkraut, pickles, green olives, fermented milk, cheese and certain sausages and in various fermented foods of plant origin.

Sauerkraut – lactic acid – 1.7%

Green olives – lactic acid – 0.9%

Fermented milk – lactic acid – 0.6 – 0.85%

Acidity of cheese usually is expressed in terms of hydrogen ion concentration, most freshly made cheeses have a P^H of about 5.0 – 5.2 and become more alkaline during curing.

Alcohol content of beer, ale, fermented fruit juices, and distilled liquors has a preservative effect.

LECTURE-16

Food Preservation by Radiation – U.V. Radiation, ionizing radiations, definition of terms, x-rays, gamma rays and cathode rays, Microwave processing. High pressure processing (pascalization)

Low-frequency, long-wavelength, low energy radiation ranges from radio waves to infrared. Conversely, the high-frequency, shorter-wavelength radiations have high quantum energies and actually excite or destroy organic compounds and microorganisms without heating the product.

Microbial destruction without the generation of high temperatures suggested the term "cold sterilization."

Radiations of higher frequencies have high energy contents and are capable of actually breaking individual molecules into ions, hence the term ionizing irradiation.

Ultraviolet Irradiation

Ultraviolet irradiation has been the most widely used in the food industry. Radiation with wavelengths near 260 nm is absorbed strongly by purines and pyrimidines and is therefore the most germicidal. Ultraviolet radiation around 200 nm is strongly absorbed by oxygen, may result in the production of ozone, and is ineffective against microorganisms.

Germicidal Lamps

The usual source of ultraviolet radiation in the food industry is from quartz mercury vapor lamps or low-pressure mercury lamps, which emit radiation at 254 nm. Radiation from these lamps includes rays in the visible range and those in the erythemic range, which have an irritating effect on skin and mucous membranes. The lamps are available in various sizes, shapes, and power. The newer types release only negligible amounts of ozone.

Factors Influencing Effectiveness

Direct rays are effective unless they come from special reflectors, and even then their effectiveness is reduced. The factors that influence the effectiveness of ultraviolet rays are as follows:

- 1. Time.** The longer the time of exposure to a given concentration, the more effective the treatment.
- 2. Intensity.** The intensity of the rays reaching an object will depend on the power of the lamp, the distance from the lamp to the object, and the kind and amount of interfering material in the path of the rays. Obviously the intensity will increase with the power of the lamp. Intensity is usually measured as microwatts per square centimeter ($\mu\text{W}/\text{cm}^2$). Within the short distance (Ex: industrial uses) the intensities of the rays vary inversely with distance from the lamp. A lamp is

about 100 times as effective in killing microorganisms at 5 in, than at 8 ft from the irradiated object. Most tests are reported from a distance of about 12 in.

Dust in the air or on the lamp reduces the effectiveness, as does too much atmospheric humidity. Over 80 percent relative humidity definitely reduces the penetration through air, but humidities below 60 percent have little effect.

3. Penetration. The nature of the object or material being irradiated has an important influence on the effectiveness of the process. Penetration is reduced even by clear water, which also exerts a protective effect on microorganisms. Dissolved mineral salts, especially of iron, and cloudiness greatly reduce the effectiveness of the rays. Even a thin layer of fatty or greasy material cuts off the rays. Therefore, the rays affect only the outer surface of most irradiated foods directly exposed to the lamp and do not penetrate to microorganisms inside the food. The lamps reduce the number of viable organisms in the air surrounding the food.

Effects on Humans and Animals

Gazing at ultraviolet lamps produces irritation of the eyes within a few seconds, and longer exposure of the skin results in erythema, or reddening. The effect on animals is usually not as marked, although the eyes, especially of chicks, may be irritated.

Action on Microorganisms

The intensity of the rays when they reach the organism, the time in which they act, and the location of the organism determine the germicidal effect. Each microorganism has a characteristic resistance to ultraviolet irradiation. This can vary with the phase of growth and the physiological state of the cell. Capsulation or clumping of bacteria increases their resistance. Bacterial spores usually take from two to five times as much exposure as the corresponding vegetative cells. Some types of pigmentation also have a protective effect. Generally, yeasts are from two to five times as resistant as bacteria, although some are easily killed. The resistance of molds is reported to be from ten to fifty times that of bacteria. The killing effect of ultraviolet rays is usually explained by the "target theory," which is described in the discussion of ionizing radiation.

Applications in the Food Industry

Examples of the successful use of these rays include treatment of water used for beverages; aging of meats; treatment of knives for slicing bread; treatment of bread and cakes; packaging of sliced bacon; sanitizing of eating utensils; prevention of growth of 'film yeast on pickle, vinegar, and sauerkraut vats; killing of spores on sugar crystals and in syrups; storage and packaging of

cheese; prevention of mold growth on walls and shelves; and treatment of air used for, or in, storage and processing rooms.

IONIZING RADIATIONS

Kinds of Ionizing Radiations

Radiation classified as ionizing includes x-rays or gamma rays, cathode or beta rays, protons, neutrons, and alpha particles. Neutrons result in residual radioactivity in foods, and protons and alpha particles have little penetration. Therefore, these rays are not practical for use in food preservation and will not be discussed.

X-rays are penetrating electromagnetic waves which are produced by bombardment of a heavy-metal target with cathode rays within an evacuated tube. They are not currently considered economical for use in the food industry.

Gamma rays are like x-rays but are emitted from by-products of atomic fission or from imitations of such by-products. Cobalt 60 and Cesium 137 have been used as sources of these rays in most experimental work thus far, with Cobalt 60 being the most promising for commercial applications.

Beta rays are streams of electrons (beta particles) emitted from radioactive material. Electrons are small, negatively charged particles of uniform mass that form part of the atom. They are deflected by magnetic and electric fields. Their penetration depends on the speed with which they hit the target. The higher the charge of the electron, the deeper its penetration.

Cathode rays are streams of electrons (beta particles) from the cathode of an evacuated tube. In practice, these electrons are accelerated by artificial means.

Definition of Terms

Before the utilization of ionizing radiations can be discussed, a few terms must be defined.

A **roentgen (r)** is the quantity of gamma or x-radiation which produces one electrostatic unit of electric charge of either sign in a cubic centimetre of air under standard conditions.

A **roentgen-equivalent-physical (rep)** is the quantity of ionizing energy which produces, per gram of tissue, an amount of ionization equivalent to a roentgen. A **megarep** is 1 million rep. One r, or 1 rep, is equivalent to the absorption of 83 to 90 erg per gram of tissue.

The **rad** now is employed chiefly as the unit of radiation dosage, being equivalent to the absorption of 100 erg per gram of irradiated material. A **megarad (Mrad)** is 1 million rad, and a **kilorad (Krad)** is 1,000 rad.

An **electronvolt (eV)** is the energy gained by an electron in moving through a potential difference of 1 volt. A meV is 1 million electronvolts.

A meV, then, is a measure of the intensity of the irradiation, and a rep is a measure of the absorbed energy that is effective within the food.

A **Gray (Gy)** equals 100 rads and is being used as a term to replace rads in some references.

Radappertization is a term used to define "radiation sterilization" which would imply high dose treatments, with the resulting product being shelf-stable.

Radurization refers to "radiation pasteurization" low-dose treatments, where the intent is to extend a product's shelf life.

Radication also is a low-dose "radiation pasteurization" treatment, but with the specific intent being the elimination of a particular pathogen.

Picowaved is a term used to label foods treated with low-level ionizing radiation.

X-Rays.

X-rays, gamma rays, and cathode rays are equally effective in sterilization for equal quantities of energy absorbed. X-rays and gamma rays have good penetration, while cathode rays have comparatively poor penetration. The greatest drawback at present to the use of x-rays in food preservation is the low efficiency and consequent high cost of their production, for only about 3 to 5 percent of the electron energy applied is used in the production of x-rays.

Gamma Rays and Cathode Rays

These two types of rays are equally effective in sterilization for equal quantities of energy absorbed and apparently produce similar changes in the food being treated.

Chief sources of gamma rays are (1) radioactive fission products of uranium and cobalt, (2) the coolant circulated in nuclear reactors, and (3) other fuel elements used to operate a nuclear reactor. Cathode rays usually are accelerated by special electrical devices. The greater this acceleration (i.e., the more meV), the deeper the penetration into the food.

Penetration

Gamma rays have good penetration, but their effectiveness decreases exponentially with depth. They are effective up to 20 cm in most foods, but this depth will depend on the time of exposure. Cathode rays, on the other hand, have poor penetration, being effective at only about 0.5 cm per meV when "cross firing," that is, irradiation from opposite sides, is employed. The absorption dose level in a material is not a uniformly decreasing fraction with depth but rather builds up to a maximum at a depth equal to about one-third of the total penetration and then decreases to zero.

Efficiency

Because Cathode rays are directional, they can be made to hit the food and therefore are used with greater efficiency than gamma rays, which are constantly emitted in all directions from the radioactive sources. Various estimates of the maximal efficiency of utilization of cathode rays range between 40 and 80 percent, depending on the shape of the irradiated material, but only a maximum of 10 to 25 percent utilization efficiency is estimated for gamma rays. Radioactive sources of gamma rays decay steadily and hence weaken with time.

Safety

The use of cathode rays presents fewer health problems than the use of gamma rays, since cathode rays are directional and less penetrating can be turned off for repair or maintenance work, and presents no hazard of radioactive materials after a fire, explosion, or other catastrophe. Gamma rays are emitted in all directions, are penetrating, are continuously emitted, and come from radioactive sources. Gamma rays require more shielding to protect workers. Tests on animals and human volunteers have not indicated any ill effects from the eating of irradiated foods.

Effects on Microorganisms

The bactericidal efficacy of a given dose of irradiation depends on the following:

- 1 The kind and species of organism:
- 2 The numbers of organisms (or spores) originally present: The more organisms there are, the less effective a given dose will be.
- 3 The composition of the food: Some constituents, e.g., proteins, catalase, and reducing substances (nitrites, sulphites, and sulfhydryl compounds), may be protective. Compounds that combine with the SH groups would be sensitizing. Products of ionization may be harmful to the organisms.
- 4 The presence or absence of oxygen. The effect of free oxygen varies with the organism, ranging from no effect to sensitization of the organism. Undesirable "side reactions," are likely are intensified in the presence of oxygen and to be less frequent in a vacuum or an atmosphere of nitrogen.
- 5 The physical state of the food during irradiation. Both moisture content and temperature affect different organisms in different ways.
- 6 The condition of the organisms. Age, temperature of growth and sporulation, and state-vegetative or spore-may affect the sensitivity of the organisms.

The type of irradiation and, within limits, the pH of the food seems to have little influence on the dose needed to inactivate the organisms.

Spores of *Clostridium botulinum*, for example, have been found to be more resistant to gamma rays than are spores of a flat sour bacterium (No. 1518) and a thermophilic anaerobe (T.A. No. 3814), although the latter two are more heat-resistant.

It is to be noted that

- (1) Humans are much more sensitive to radiations than are microorganisms.
- (2) Bacterial spores are considerably more resistant than are vegetative cells.
- (3) Gram-negative bacteria are, in general, less resistant than are gram-positive ones.
- (4) Yeasts and molds vary considerably, but some are more resistant than most bacteria.

For example, yeast *Candida krusei* is as resistant as many bacterial spores. Certain microorganisms are much more resistant than anticipated. For example, a radiation-resistant micrococcus resembling *Micrococcus roseus* has been found in irradiated meat. *Microbacterium* species in meat have been found to be especially resistant.

The irradiated microorganisms are destroyed by passage of an ionizing particle or quantum of energy through, or in close proximity to, a sensitive portion of the cell, causing a direct "hit" on this target, ionization in this sensitive region, and subsequent death of the organism (this is called the target theory). It is assumed also that much of the germicidal effect results from ionization of the surroundings, especially of water, to yield free radicals, some of which may be oxidizing or reducing and therefore helpful in the destruction of the organisms. Irradiation also may cause mutations in the organisms present.

Effects on Foods

Radiation doses heavy enough to affect sterilization have been found to produce undesirable "side reactions," or secondary changes, in many kinds of foods, causing undesirable colors, odors, tastes, or even physical properties.

Some of the changes produced in foods by sterilizing doses of radiation include

- (1) In meat, a rise in pH, destruction of glutathione, and an increase in carbonyl compounds, hydrogen sulfide, and methyl mercaptan.
- (2) In fats and lipids, destruction of natural antioxidants, oxidation followed by partial polymerization, and increase in carbonyl compounds.
- (3) In vitamins, reduction in most foods of levels of thiamine, pyridoxine, and vitamins B₁₂, C, D, E, and K: riboflavin and niacin are fairly stable. The lower the dosage of irradiation, of course, the less frequent the undesirable effects on the food. Destruction of many of the food

enzymes requires five to ten times the dosage of rays needed to kill all the microorganisms. Enzyme action continues after all microorganisms have been destroyed unless a special blanching treatment has preceded irradiation.

The chief effect on the healthfulness of foods is the destruction of vitamins.

The general nutrition of an irradiated food would be as good as that of a food processed by other means to achieve the same shelf stability. There is no indication of production of radioactivity with electron beams below 11 me V or with gamma rays from Cobalt 60.

Applications

Currently food irradiation has been approved only in a very limited way in the United States. Low-level irradiation (1 kiloGray) can be used on fresh fruits and vegetables to kill insects and to inhibit spoilage.

Dry or dehydrated vegetables (herbs and spices) can be irradiated at up to 30 kiloGray to kill insects and bacteria.

MICROWAVE PROCESSING

Microwave heating and processing of foods is becoming increasingly popular, particularly at the consumer level. Microwaves are electromagnetic waves between infrared and radio waves. Specific frequencies are usually at either 915 megacycles or 2,450 megacycles. The energy or heat produced by microwaves as they pass through a food is a result of the extremely rapid oscillation of the food molecules to align themselves with the electromagnetic field being produced. This rapid oscillation, or intermolecule friction, generates heat. The preservative effect of microwaves or the bactericidal effect produced is really a function of the heat that is generated. But the destruction of microorganisms is due to the heat produced by the excitation of food molecules.

High Hydrostatic Pressure (HHP). (Pascalization)

Also called as pascalization. This technique is applied to foods, which can be liquid or solid, packaged or unpackaged, to high pressure (which varies depending upon application) usually for 5 minutes or less. HHP can be used on many foods such as raw and cooked meats, fish and shellfish, fruit and vegetable products, cheeses, salads, dips, grains and grain products, and liquids including juices, sauces, and soups. The high pressure does not destroy the food. Microorganisms living on the surface and in the interior of the food are inactivated. Inactivation is accomplished by affecting the molecular structure of chemical compounds necessary for metabolic metabolism in the microorganisms. HHP is equally effective on molds, bacteria, viruses, and parasites.

LECTURE-17

Microbiology of milk and milk products. Contamination, preservation, pasteurization and ultra pasteurization, vat pasteurization. Vaccination use of low temperatures, freezing, drying etc.,

Dairy products include market milk and cream, butter, frozen desserts, cheese, fermented milks, and condensed and dried milk products.

CONTAMINATION

On the farm:

Milk contains relatively few bacteria when it leaves the udder of a healthy cow, and generally these bacteria do not grow in milk under the usual conditions of handling. However, micrococci and streptococci have been recovered from aseptically drawn milk. During the normal milking operation, milk is subject to contamination from the animal, especially the exterior of the udder and adjacent areas.

Bacteria found in manure, soil, and water may enter from this source. Such contamination is reduced by clipping the cow, especially the flanks and udder, grooming the cow, and washing the udder with water or a germicidal solution before milking. Contamination of the cow with soil, water, and manure is reduced by paving and draining barnyards, keeping cows from stagnant pools, and cleaning manure from the barns or milking parlors.

Probably the two most significant sources of contamination are dairy utensils and milk-contact surfaces, including the milk pail or milking machines, strainers, milk cans or pipelines, and the bulk-milk cooler.

Undesirable bacteria from these sources include lactic streptococci, coliform bacteria, psychrotrophic gram-negative rods, and thermotolerant, those which survive pasteurization, e.g., micrococci, enterococci, bacilli, and brevibacteria. In general, these bacteria grow well in milk and hence endanger its keeping quality.

Other possible sources of contamination are the hands and arms of the milker or dairy workers, the air of the barn or milking parlor, and flies. Normally these sources would contribute very few bacteria, but they might be a source of pathogens or spoilage microorganisms. The quality of the farm water supply used in the milking parlor for cleaning, rinsing, etc., will have some effect on the quality of the milk.

The numbers of bacteria per milliliter of milk added from the various sources depends on the care taken to avoid contamination. For example, the exterior of the cow contributes

comparatively few organisms if precautions are taken and a milking machine is used, but under very poor conditions thousands per milliliter could enter the milk.

In transit and at the manufacturing level

Other sources of contamination after the milk leaves the farm include the tanker truck, transfer pipes, sampling utensils, and the equipment at the market-milk plant, cheese factory, condensery, or other processing plant.

The most significant sources of contamination are the milk-contact surfaces. Pipelines, vats, tanks, pumps, valves, separators, clarifiers, homogenizers, coolers, strainers, stirrers, and fillers may serve as possible sources of bacteria. The amount or level of contamination from each of these sources depends on cleaning and sanitizing methods.

The employees, particularly their hands and arms, are a possible source of contamination and pathogens. The paper stock used for packaging fluid milk is also an important source of contamination.

The number and types of organisms in milk or other dairy products may be increased either by contamination or by growth of the organisms already present. Methods of production, handling, storage, and manufacture are designed to prevent both.

PRESERVATION:

Asepsis

The prevention of the contamination of milk is important in its preservation. Keeping quality is usually improved when smaller numbers of microorganisms are present, especially those which grow readily in milk.

Cleaning of dairy utensils, cow, milk-contact surfaces like pipelines, vats, tanks, pumps, valves, separators, clarifiers, homogenizers, coolers, strainers, stirrers, and fillers is maintained to reduce the microbial contamination.

Since numbers of bacteria in milk are indicative of the sanitary precautions and careful handling employed during the production.

Packaging serves to keep microorganisms from bottled milk, fermented milks, packaged butter, canned milk, dry milk, and packaged cheese, and so do coatings of plastic, wax, or other protective substances on finished cheeses. The bacteriological quality of the paper stock used in the fabrication of paper milk cartons has been examined. Normally the packaging material contributes very little to the total microbial load in the finished product.

Removal of Microorganisms:

After microorganisms have entered milk, it is difficult to remove them effectively. The process of centrifugation, as in clarifying or separating, will remove some microorganisms from milk.

High-speed centrifugation (at 10,000 g) removes about 99 percent of the spores and more than half of the vegetative cells of bacteria plus some protein.

Bactofugation: The centrifugal procedure used for removing bacteria from milk, known as bactofugation. It is not used extensively on a commercial basis. Molds can be removed physically from the surface of some kinds of cheese during the curing process by scraping or periodic washing.

USE OF HEAT

Pasteurization and Ultrapasteurization:

Because milk and cream are so readily changed by heat, the mild heat treatment called pasteurization is used for their preservation. Heat treatments to milk increase its shelf life. Pasteurization became a necessary safeguard to avoid food borne illness caused by contaminated milk.

The objectives of market-milk pasteurization are to

- (1) Kill all the pathogens that may enter the milk and be transmitted to people.
- (2) Improve the keeping quality of milk.

Ideally, this heat treatment should be accomplished without deleteriously affecting the flavor, appearance, nutritional properties, or creaming of the milk.

When milk is pasteurized for the manufacture of cheese or cream is pasteurized for making butter, a third objective is to destroy microorganisms that would interfere with the activities of desirable organisms, such as the starter culture, or cause inferiority or spoilage of the product. The heat treatment does not harm the curdling properties of the milk. The heat treatment of cream also destroys lipases that may cause deterioration of butter during storage.

The first widely used pasteurization process for milk involved heating the milk in large tanks or vats to 60⁰ C for at least 20 min. This holding method was subsequently changed to 61.7⁰ C for 30 min. and finally to 62.8⁰ C for 30 min to eliminate *Coxiella burnetii*, a rickettsia responsible for Q fever which can be transmitted in milk. (This temperature kills *Mycobacterium tuberculosis* but not *Coxiella burnetti*, hence pasteurization temperature was changed from 61.7⁰C to 62.8⁰C). This was not a continuous process and was referred to as vat pasteurization.

HTST: (High temperature and Short time): The use of plate heat exchangers and a continuous operation involves the high-temperature-short-time (HTST) pasteurization process at a temperature of at least 72°C for at least 15 sec. The HTST system is the most widely used commercial pasteurization process today.

LTLT (Low temperature long time): It involves holding the milk for 62.8°C for 30 minutes

The main objective is to decrease total microbial load and thereby increase the shelf life of the product. Recent disease outbreaks attributed to milk and other dairy products have implicated *Listeria monocytogenes* as the causative agent

Heat-treatment processes in excess of pasteurization for milk and milk products have been designated as very high temperature (VHT) systems and ultrahigh temperature (UHT) systems. There does not appear to be a precise definition for a VHT system.

Ultrahigh temperature (UHT): According to the International Dairy Federation, UHT processes usually refer to pasteurization techniques with temperatures of at least 130°C in a continuous flow, with holding times of approximately 2 sec to be labeled "ultrapasteurized." UHT systems have been more extensively used in Europe than in the United States for fluid milk.

Current federal standards of identity stipulate that a product must have been heated to 137.8°C or above and held there for at least 2 sec to be labeled "ultrapasteurized."

Products such as cream for whipping, coffee cream, and half and half are processed by UHT systems. The current drawback to UHT systems is that the severe heating needed could affect or alter the nutritive and organoleptic properties of the product.

The most popular of the UHT systems are the direct-heating methods like steam injection technique and steam infusion technique.

1. Steam-injection technique: It includes a steam is injected into milk process, referred to as a steam-injection technique.

2. Steam infusion technique: It includes milk is injected into the steam process, referred as Steam infusion technique.

In both these systems the added steam or excess water is removed following the heat treatment in a sterile vacuum chamber. The combination of this type of heat treatment with aseptic packaging results in a category of products usually referred to as "sterilized milk" or "sterilized cream." This designation does not imply absolute sterility but a sterile product in the commercial sense. These products have an extremely long shelf life around 6 weeks.

The efficiency of milk pasteurization or the percentage of reduction of numbers of microorganisms in milk during pasteurization depends on

- (1) The temperature of pasteurization.
- (2) The holding time.
- (3) The total numbers of bacteria
- (4) The proportion of the total microbial load that are sporeformers or thermophilic organisms.

In general, the conventional HTST system may reduce the number of microorganisms in milk by at least 90 to 99 percent. Following pasteurization, milk is cooled rapidly to at least 7.2°C or less. The shelf life of pasteurized milk depends on the temperature of storage and the numbers and types of microorganisms surviving the pasteurization process.

Cream for butter making is given a greater heat treatment during pasteurization than is market cream. Heating is at 71.1°C or above for 30 min by the holding method or 87.7 to 93.3°C for a few seconds by the HTST method.

Cream is more protective to organisms than is milk, and cream for butter making is likely to contain a higher population of microorganisms than most lots of milk.

Vacreation: Rapid heating of cream is accomplished by injecting steam or by a combination of steam injection and evacuation in a process known as vacreation.

Conventional pasteurization should kill all yeasts and molds and most vegetative cells of bacteria in the milk.

The surviving bacteria, termed thermophilics, belong to a number of different groups of bacteria, of which only a few of the more important ones will be mentioned. Most important of the non-spore-forming bacteria are

- (1) The high-temperature lactics, e.g., the enterococci; *Streptococcus thermophilus*, high-temperature lactobacilli, such as *Lactobacillus bulgaricus*, and *L. lactis*; and species of *Microbacterium*.
- (2) Certain species of *Micrococcus*.

Some species of *Streptococcus* and *Lactobacillus* are thermophilic as well as thermophilics. The spore-forming thermophilics fall into two main groups:

- (1) Species of *Bacillus*, i.e., aerobic to facultative spore-forming bacilli, of which *B. cereus* (proteolytic) usually is the most numerous but *B. licheniformis*, *B. subtilis* (proteolytic), *Bacillus coagulans* (thermophilic), *B. polymyxa* (gas-forming), and other species sometimes are of importance.

(2) Species of *Clostridium*, anaerobic, spore-forming rods, some of which are saccharolytic, e.g., *C. butyricum*, and others proteolytic and saccharolytic, e.g., *C. sporogenes*. Most of those growing in milk also form gas.

(3) Other miscellaneous bacteria may survive pasteurization but do not grow well in milk.

Steam under Pressure:

Evaporated milk is canned and then heat-processed by steam under pressure, often with accompanying rolling or agitation. The fore warming of the milk at about 93 to 100°C or higher before evaporation kills all but the more resistant bacterial spores. Sealed cans of evaporated milk are processed at about 115 to 118 C for 14 to 18 min, which results in a commercially sterile product.

USE OF LOW TEMPERATURES

With the exception of canned milk and dry milk, most dairy products require the use of low temperatures as one factor in their preservation; often this is the most important factor.

Refrigerated Storage:

For the production of milk of good quality it is essential that it be cooled promptly after it is withdrawn from the cow.

The Grade A Pasteurized Milk Ordinance of the United States Public Health Service stipulates that Grade A raw milk for pasteurization shall be cooled to 10°C or less within 2 hr after being drawn and kept that cold until processed.

Newly pasteurized milk is to be cooled to 7.2° C or less and maintained there. It is preferable, of course, to cool it to temperatures well below 7.2 C. For example, the bulk-milk coolers on farms can rapidly cool milk to 3.0 to 4.5°C or lower and hold that temperature, except for short periods when fresh milk is entering, and then the temperature usually does not exceed 7.2°C.

Milk is held at refrigeration temperatures during storage on the farm, in the truck or tank during transportation to the plant or receiving station, and during storage there. Refrigeration temperatures are recommended for the milk or related products during storage in the plant or in the retail market and during delivery and in the home or restaurant until consumption.

Fermented milks and unripened cheeses are chilled after their manufacture and kept chilled until they reach the consumer.

Most kinds of ripened cheese also are stored at chilling temperatures after their ripening is complete.

Freezing

Ice cream and other frozen dairy desserts are frozen as part of the manufacturing process and are stored at low temperatures in the frozen state, where microbial multiplication is impossible. The microbial content of the ingredients like milk, cream, sugar, eggs, stabilizers, and flavoring and coloring materials-along with contamination picked up during processing will determine the numbers and kinds of microorganisms in the mix and the microbial content after pasteurization of the mix and freezing.

Pasteurization reduces numbers and kinds of microorganisms, but freezing kills relatively few of the organisms, and storage in the frozen state permits survival of most of the microorganisms for long periods.

Butter in storage is held at -17 to -18°C or lower, where no microbial growth can take place. Frozen cream is stored in considerable amounts at a similar temperature.

Milk, concentrated to one-third its volume, can be frozen at -17 to -18°C and stored at -23 to -24°C or lower and can be held for several weeks without deterioration. Frozen milk can be concentrated by freeze-drying methods. Pasteurized whole milk has been frozen at about -28 to -29°C and shipped and stored in the frozen state.

DRYING

The reduction in moisture and consequent increase in the concentration of dissolved substances in liquid condensed milk products inhibits the growth of some kinds of bacteria.

Condensed Products:

Evaporated milk is made by removing about 60 percent of the water from whole milk, so that about 11.5 percent lactose would be in solution, plus twice the amount of soluble inorganic salts in whole milk. This high concentration of sugar is inhibitory to the growth of some bacteria and prevents the growth of some bacteria.

Bulk condensed milk is more condensed than evaporated milk and is a still poorer culture medium for organisms not tolerant of high sugar concentrations. Condensed whey, called **whey semisolids**, is another concentrated dairy product, as is condensed buttermilk, called **Semisolid buttermilk**, which has its concentration of acid and other solutes increased by the condensation process.

High concentrations of sugar and the increased percentage of soluble inorganic salts tie up the moisture, making it unavailable to any microorganism except osmophilic microorganisms. Therefore, drying, both by removal of water and by tying it up, is a main preservative factor.

Dry Products

Among the dairy products prepared in the dry form are milk, skim milk, cream, whey, buttermilk, ice cream mix, and malted milk.

Usually the milk is preheated before drying (to 65 to 85 C for the roller process and to 68.8 to 93.3 C for the spray process). This preheating process pasteurizes the milk and kills the less heat-resistant microorganisms.

Some of the drying processes involve an instantization step, in which the dry powder is wetted and then redried. In this process the product is actually exposed to contact with the air at nine different stages. A large salmonellosis outbreak involving powdered milk was shown to have resulted from air contamination during this instantizing process.

The microbial content of the heat-dried dairy product depends on the content of the liquid product to be dried, the temperature and time of preheating, the evaporation process, contamination and growth in storage tanks and pipes.

The method of drying, and contamination from air sources. Preheating kills organisms as pasteurization would and hence destroys all but the thermodurics. Evaporation, especially a continuous process, may result in increases in thermodurics and bacteria that are thermophilic.

The high temperature of the roller process without vacuum destroys almost all organisms except bacterial spores.

Added Preservatives:

The addition of preservatives to dairy products is permitted only to a limited extent. The use of sorbic or propionic acid or one of their salts is permitted in cottage cheese, yogurt, and some of the hard cheeses and processed cheeses. The primary objective in adding a preservative to hard cheeses or preserved cheeses is to prevent the surface growth of molds.

Added sugar acts as a preservative of sweetened condensed milk; it reduces the a_w , thereby making moisture unavailable to microorganisms.

Sodium chloride or common salt is added in the manufacture of various kinds of cheese, but usually it is more for flavor or for controlling the growth of microorganisms during manufacturing and curing than for preservation of the finished product.

The sodium chloride in salted butter is in a concentration in a liquid phase sufficient to prevent the growth of many bacteria and to cause a decrease in the number of those which are not salt-tolerant.

In butter with 16.34 percent moisture and 2.35 percent salt, the water phase would be about 12.6 percent salt brine.

Cheese is smoked primarily for the addition of flavor, although the drying, especially of the rind, and the chemical preservatives from the smoke may improve the keeping quality. Mold spoilage of cheese is usually delayed or prevented when sorbic acid, sorbates, propionic acid, or propionates are added or incorporated in the wrapper.

The addition of hydrogen peroxide combined with a mild heat treatment has been used for pasteurization of milk for certain kinds of cheese (e.g., Swiss and Cheddar). The excess peroxide is usually destroyed by added catalase.

Developed Preservatives

Most fermented products are microbiologically more stable or have a longer shelf life than the initial substrate. Fermented milks and cheese are preserved partly by the developed acidity produced by the bacterial culture and therefore have a longer shelf life than fluid milk.

Other Methods

The treatment of milk with ultraviolet rays is not used in the preservation of milk because only a thin layer of milk can be successfully irradiated and, unless great care is taken, a "burnt" flavor will result. Other uses of ultraviolet light in the dairy industry include irradiation of rooms to reduce the numbers of microorganisms in the air in processing rooms where sweetened condensed milk is being prepared or cut cheese is being packaged and in cheese curing rooms.

The rays inhibit mold growth on the sides of the curing cheese exposed to them directly but are not effective on the shaded side. Ultraviolet light is also used in storage tanks containing liquid sugar or corn-sirup solids (ingredients in ice cream) to prevent mold growth on the surface.

Several preservative factors are involved in the preservation of milk and its products.

1. Milk for market sale or for the manufacture of dairy products is produced as aseptically as practicable, is cooled promptly, and is kept chilled.
2. It is pasteurized, and then packaged to keep out microorganisms. Cream is treated similarly.
3. Fermented milks owe most of their keeping qualities to the acid formed during the fermentation but require chilling and packaging for their preservation.
4. Butter is preserved primarily by low temperatures, chilling for short-time storage or freezing temperatures for long-time storage. The low moisture and the salt content also aid in the preservation, as does packaging or sealing to prevent contamination.
5. Cheese is preserved by the acidity produced during its making, by chilling, and by impervious rinds or by packaging.

6. Dry milk, if properly prepared, has too little moisture for microbial growth but requires packaging to prevent contamination. Evaporated milk is processed by steam under pressure to kill all or most microorganisms and is sealed in cans to keep out contaminants.
7. Sweetened condensed milk undergoes pasteurization during its preparation, contains a high concentration of sugars, and is protected by the sealed can.

LECTURE-18

Spoilage of milk and cream, gas production proteolysis, ropiness, changes in milk fat. Alkali production. Flavor changes & colour changes.

SPOILAGE

1. Milk and Cream – Gas production, Proteolysis, Ropiness, Changes in Milk fat, Alkali production, Flavour changes, Color Changes, Spoilage of milk at different temperatures
2. Condensed & Dairy Milk products – Bulk condensed milk, Evaporated milk, Sweetened Condensed Milk,
3. Frozen desserts
4. Butter – Flavor defects, Color defects

1. MILK AND CREAM:

Milk is an excellent culture medium for many kinds of microorganisms, being high in moisture, nearly neutral in pH, and rich in microbial foods. A plentiful supply of food energy is present in the form of milk sugar (lactose), butterfat, citrate, and nitrogenous compounds in many forms (proteins, amino acids, ammonia, urea, and other compounds), and the accessory foods and minerals required by microorganisms are available. Some inhibitory substances (lactoperoxidase and agglutinins) are present in freshly drawn milk but soon become comparatively ineffective.

Because of the fermentable sugar, an acid fermentation by bacteria occurs under ordinary conditions in raw milk, but other changes may take place if conditions are unfavorable to the acid formers or if they are absent. With a trend toward higher pasteurization temperatures, the spoilage flora of pasteurized milk is more frequently becoming heat-resistant, spore-forming bacilli which can be psychrotrophic.

When milk sours, it usually is considered spoiled, especially if it curdles. The evidences of acid formation are first a sour flavor and then coagulation of the milk to give a solid jelly like curd or a weaker curd that releases clear whey. The lactic acid fermentation is most likely to take place in raw milk held at room temperatures.

In raw milk at temperatures from 10 to 37°C, *Streptococcus lactis* is most likely to cause the souring, with possibly some growth of coliform bacteria, enterococci, lactobacilli, and micrococci. At higher temperatures, e.g., from 37 to 50°C, *S. thermophilus* and *S. faecalis* may produce about 1 percent acid and be followed by lactobacilli, such as *Lactobacillus bulgaricus*, which will produce more acid. Some of the lactobacilli can grow at temperatures above 50°C but

produce less acid there. Thermophilic bacteria can grow at still higher temperatures, e.g., *Lactobacillus thermophilus*. Little formation of acid takes place in milk held at temperatures near freezing, but proteolysis may take place.

The pasteurization of milk kills the more active acid-forming bacteria but may permit the survival of heat-resistant lactics (e.g., enterococci, *Streptococcus thermophilus*, and lactobacilli), which will cause a lactic acid fermentation if the subsequent storage temperature is high enough.

Many bacteria other than those termed lactics can cause an acid fermentation in milk, especially if conditions are unfavorable for the lactic acid bacteria. The coliform bacteria produce some lactic acid and considerable amounts of volatile products, such as hydrogen, carbon dioxide, acetic acid, formic acid, alcohol, etc. Species of *Micrococcus*, *Microbacterium*, and *Bacillus* can produce acid in milk, mostly lactic, but ordinarily cannot compete with the lactics.

Butyric acid may be produced in milk by action of *Clostridium* spp. under conditions that prevent or inhibit the normal lactic acid formation. Heat treatment destroys all vegetative cells of bacteria but allows the survival of spores of *Clostridium*, milk may undergo butyric acid fermentation with the production of hydrogen and carbon dioxide gas.

Gas Production:

Gas production by bacteria usually is accompanied by acid formation and it is undesirable in milk and milk products. The chief gas formers are the coliform bacteria, *Clostridium* spp., gas-forming *Bacillus* species that yield both hydrogen and carbon dioxide.

Yeasts, propionics, and hetero fermentative lactics produce only carbon dioxide.

The production of gas in milk is evidenced by foam at the top if the milk is liquid and is super saturated with the gas, by gas bubbles caught in the curd or furrowing it, by floating curd containing gas bubbles, or by a ripping apart of the curd by rapid gas production, causing the so-called stormy fermentation of milk.

In raw milk the coliform bacteria are most apt to be the main gas formers. Heterofermentative lactics also may produce gas, but usually not enough to be evident in the milk. Yeasts (lactose-fermenting) usually are absent or in low numbers in milk and do not compete well with the bacteria.

Gas-forming *Clostridium* and *Bacillus* do not compete well with acid formers at higher temperatures but may function if the acid formers are absent or comparatively inactive. Milk heated at pasteurizing temperatures or above, the chief acid formers will be killed, the

spores of *Clostridium* and *Bacillus* species will survive, and gas formation by the spore formers may take place.

Propionic acid-forming bacteria are not active in milk but form gas (carbon dioxide) in cheese.

Proteolysis:

The hydrolysis of milk proteins by microorganisms usually is accompanied by the production of a bitter flavor caused by some of the peptides released.

Proteolysis is favored by 1. Storage at a low temperature. 2. by the destruction of lactics and other acid formers by heat. 3. By the destruction of formed acid in the milk by molds and film yeasts. 4. The neutralization of acids by products of other organisms.

The types of change produced by proteolytic microorganisms include

- (1) Acid proteolysis, in which acid production and proteolysis occur together.
- (2) Proteolysis with little acidity or even with alkalinity.
- (3) Sweet curdling, caused by rennin like enzymes of the bacteria at an early stage of proteolysis.
- (4) Slow proteolysis by intracellular enzymes of bacteria after their autolysis.
- (5) Residual proteolytic activity of heat-stable proteinase.

For example, *Pseudomonas fluorescens* produces a proteinase that will survive pasteurization even though the bacterium does not.

Acid proteolysis causes the production of a shrunken curd and the expression of much whey. This is followed by a slow digestion of the curd, which changes in appearance from opaqueness to translucency and may be completely dissolved by some kinds of bacteria.

Acid proteolysis may be caused by several species of *Micrococcus*, some of which grow in the udder of the cow and cause acid proteolysis of aseptically drawn milk.

One of the intestinal streptococci or enterococci, *Streptococcus faecalis* var. *liquefaciens*, is a lactic acid organism that also is actively proteolytic. It is thermotolerant, like the other enterococci, and may cause acid proteolysis in pasteurized milk. Spores of lactose-fermenting, proteolytic strains of some species of *Bacillus* can survive pasteurization or a more rigorous heat treatment of milk and cause acid proteolysis.

Proteolytic bacteria are the species of *Micrococcus*, *Alcaligenes*, *Pseudomonas*, *Proteus*, *Flavobacterium*, and *Serratia*, all of which are genera of non-spore-forming bacteria, and of the genera *Bacillus* and *Clostridium* of the spore formers. Some species of the genera *Micrococcus*, *Pseudomonas*, *Alcaligenes*, *Flavobacterium*, and *Bacillus* can grow at low temperatures and

hence are likely to cause some proteolysis and/or bitterness of milk held at chilling temperatures. *Bacillus cereus* has been implicated in sweet curdling of pasteurized milk.

This spoilage condition is becoming more common in milk because of

- (1) Higher pasteurization temperatures.
- (2) Psychrotrophic capacity of some bacilli.
- (3) Longer holding or shelf-life times.

Slow proteolysis by intracellular enzymes of bacteria after their autolysis is of no significance in milk under ordinary circumstances but is significant when a long time is allowed for their action, as in curing cheese or in products with a long shelf life. .

Ropiness:

Ropiness and sliminess can occur in milk, cream, or whey but are important mostly in market milk and cream.

Nonbacterial ropiness or sliminess may be due to

- (1) Stringiness caused by mastitis and in particular by fibrin and leukocytes from the cow's blood (in contrast to ropiness produced by bacteria, it is present when the milk is drawn, not developed during holding of the milk).
- (2) Sliminess resulting from the thickness of cream, e.g., at the top of a bottle.
- (3) Stringiness due to thin films of casein or lactalbumin during cooling, as sometimes is observed on surface coolers. This effect is only temporary.

Bacterial ropiness is caused by slimy capsular material from the cells, usually gums or mucins, and develops best at low storage temperatures. The ropiness decreases as the acidity of the milk or cream increases.

There are two main types of bacterial ropiness, one in which the milk is most ropy at the top and the other in which the milk becomes ropy throughout.

Surface ropiness is caused most often by *Alcaligenes viscolactis*, an organism chiefly from water or soil that can grow fairly well in the vicinity of 10°C. Some of the thermophilic micrococci, e.g., *Micrococcus freudenreichii*, can cause surface ropiness.

Ropiness throughout the milk may be caused by any of a number of kinds of bacteria

1 *Enterobacter aerogenes*, *E. cloacae*, *Klebsiella oxytoca*, and rarely *Escherichia coli*. Ropiness caused by *Enterobacter* usually is worse near the top of the milk.

2. Certain strains of some of the common species of lactic acid bacteria. *Streptococcus lactis* var. *hollandicus* causes ropiness in milk, and is used in making Scandinavian fermented milk. *Lactobacillus casei*, *L. bulgaricus*, and *L. plantarum* occasionally produce ropiness, as do strains

of *Streptococcus cremoris*. Most of these lactic bacteria can grow in long chains, a characteristic that supposedly contributes to the stringy condition of the milk.

3. Miscellaneous other bacteria among the alkali formers, micrococci, streptococci, and bacilli. Ordinarily these bacteria would be suppressed by the acid formers.

Since the sources of the bacteria causing ropiness are water, manure, utensils, and feed, the reduction or elimination of contamination from these sources helps prevent ropiness. Adequate pasteurization of milk readily destroys most of these kinds of bacteria.

Changes in Milk fat:

Milk fat may be decomposed by various bacteria, yeasts, and molds that do not constitute distinct groups on the basis of other characteristics. The bacteria are for the most part aerobic or facultative, proteolytic, and non-acid-forming. The following changes in the milk fat take place:

1. Oxidation of the unsaturated fatty acids, which, coupled with other decomposition, yields aldehydes, acids, and ketones and results in tallowy odors and tastes. The reaction is favored by metals, sunlight, and oxidizing microorganisms.
2. Hydrolysis of the butterfat to fatty acids and glycerol by the enzyme lipase. The lipase may have been in the original milk or may be microbial.
3. Combined oxidation and hydrolysis to produce rancidity.

Species of lipase-forming bacteria are found in many of the bacterial genera, e.g., *Pseudomonas*, *Proteus*, *Alcaligenes*, *Bacillus*, *Micrococcus*, *Clostridium*, and others. Many of the molds and some species of yeasts are lipolytic.

Pseudomonas fragi and *Staphylococcus aureus* produce fairly heat-resistant lipases which may survive pasteurization if present in the raw milk.

Alkali Production:

The group of alkali formers includes bacteria which cause an alkaline reaction in milk without any evidence of proteolysis. The alkaline reaction may result from the formation of ammonia, as from urea, or of carbonates, as from organic acids such as citric acid. Most of these bacteria grow from moderate to low temperatures, and many can survive pasteurization. Examples of alkali formers are *Pseudomonas fluorescens* and *A. viscolactis*.

Flavor Changes

As drawn from the cow, milk may be abnormal in flavor because of the individual cow, mastitis, the stage of lactation of the cow, or feed.

Some of the off-flavors caused by microorganisms are described as follows.

1. Sour or Acid Flavor: The acidity may be described as

"clean," as produced by *Streptococcus lactis* and other lactic.

"aromatic;" when lactic streptococci and aroma-forming *Leuconostoc* species are growing together.

"sharp," when appreciable amounts of volatile fatty acids (formic, acetic, or butyric) are produced by coliform bacteria, *Clostridium* spp., and other organisms.

Clean and aromatic flavors are desired in fermented milk products, but sharp flavors are undesirable. .

2. Bitter Flavors: Bitterness usually results from proteolysis but may follow lipolysis or even fermentation of lactose. Milk from cows late in their lactation period sometimes is slightly bitter. Other organisms causing bitterness are certain strains of coliform bacteria and of asporogenous yeasts.

Some cocci cause very bitter milk

Actinomycetes sometimes give bitter musty flavors.

3. Burnt or Caramel Flavor:

Certain strains of *Streptococcus lactis* var. *maltigenes* produce this flavor, which resembles the cooked flavor of overheated milk.

4. Miscellaneous Other Flavors: Other flavors that are found less commonly are:

A barny flavor by *Enterobacter oxytocum*

Soapiness by ammonia formers like *Pseudomonas sapolactica*

A turnip like flavor by *Escherichia coli* and *P.fluorescens*

A malty flavor by yellow micrococci from the udder

Fruity flavor by *P. fragi*

A potato like flavor by *P. mucidolens*,

Fishiness by *Aeromonas hydrophila* or various cocci that produce trimethylamine from lecithin

Earthy or musty flavors by actinomycetes

Fruity, ester like, and alcoholic flavors by yeasts

An amyl alcohol flavor by white and orange micrococci

Putrefaction by species of *Clostridium*, *P. putrefaciens*, and other putrefactive bacteria.

Other flavors have been termed unclean, stale, astringent, oily, weedy, caroty, etc.

Inadequately cooled raw milk that has been held in a tightly covered can so that volatile products of bacterial metabolism have collected above the milk has an undesirable odor that varies in nature. Milk in this condition is termed **smothered**.

Color Changes

Color changes caused by microorganisms may occur along with other changes. The color may be due to the surface growth of pigmented bacteria or molds in the form of a scum or ring or may be present throughout the milk.

1. Blue Milk: *Pseudomonas syncyanea* produces a bluish-gray to brownish color in milk in pure culture but when growing with an acid former like *Streptococcus lactis* causes a deep-blue color. This defect and the blue colour produced by actinomycetes or species of the mold *Geotrichum* are rare.

2. Yellow Milk: *Pseudomonas synxantha* may cause a yellow color in the cream layer of milk, coincident to lipolysis and proteolysis. Species of *Flavobacterium* can also give yellowness.

3. Red Milk: Red milk usually is caused by species of *Serratia*, e.g., *S. marcescens*, but is rare because other bacteria ordinarily outgrow the red-pigmented species. *Brevibacterium erythrogenes* produces a red layer at the top of the milk, followed by proteolysis. *Micrococcus roseus* may grow and produce red sediment, and yeast may produce pink or red colonies on the surface of sour milk or cream. Blood in milk will give it a red color. The red blood cells settle out or can be centrifuged out.

4. Brown Milk: A brown color may result from *Pseudomonas putrefaciens* or by the enzymic oxidation of tyrosine by *P. fluorescens*.

LECTURE-19

Spoilage of milk at different temperatures. Condensed and dry milk products. Flavour defects, color defects.

Spoilage of Milk at Different Temperatures

At any given storage temperature most samples of raw milk undergo a typical series of changes caused by a succession of microorganisms.

At refrigeration temperatures, proteolysis may be initiated by psychrotrophic bacteria such as *Pseudomonas*, and molds may then appear. At room temperatures, an acid fermentation is most probable, first by lactic streptococci and coliform bacteria and then by the acid-tolerant lactobacilli. Then molds or film yeasts on the surface lower the acidity, permitting the formation of more acid. Eventually, when most of the acid has been destroyed, proteolytic or putrefactive bacteria complete the decomposition.

Pasteurization as applied commercially in HTST systems kills yeasts, molds, most psychrotrophic bacteria, the coliforms, and rapid acid producers such as *Streptococcus lactis*. The spoilage of pasteurized milk then depends on:

1. The bacteria that survive pasteurization, the "thermodurics" and spore formers.
2. The bacteria that enter the milk following pasteurization, post pasteurization contamination from equipment, filling operation, and the package itself.
3. The possible presence of heat-resistant residual microbial enzymes
4. The temperature of storage.

2. CONDENSED AND DRY MILK PRODUCTS

Condensed and dry milk products include evaporated milk (unsweetened), bulk condensed milk, frozen milk, sweetened condensed milk, condensed whey or buttermilk, and condensed sour skim milk and dry milk. The quality of all of these products depends on the quality of the starting material dried or condensed, since defects in the raw material carryover to the condensed or dried product. All the condensed products have a fairly high concentration of solutes that inhibits the growth of some bacteria. Dry milk is so low in moisture that it offers no microbial spoilage problems when properly handled. Moisture contents over 8 percent might permit some mold growth. The only spoilage of condensed butter milk and sour skim milk is by molds when the surface is exposed to air. The high concentration of acid and solutes prevents the growth of bacteria or yeasts.

Bulk Condensed Milk

The fore warming temperatures employed in the making of plain condensed milk are equivalent to pasteurization, and the evaporating process is at a temperature low enough to permit the growth of thermophiles. Therefore, although refrigerated, this product has only a short storage life and is subject to spoilage by thermophilic bacteria that tolerate the increased concentration of solutes in the condensed product.

Evaporated Milk

Unsweetened evaporated milk is canned and heat-processed under steam pressure to destroy all the microorganisms present. Spoilage can take place only when the heat process is inadequate or defects in the can permit the entrance of organisms. Bacterial spores that survive the heat process may be the cause of can swelling, milk coagulation, or development of a bitter flavor.

Swelling of the can is caused primarily by gas-forming anaerobic spore formers (*Clostridium*), although over filling of the can with cold milk may cause swelling. Acid constituents of milk acting on the iron of the can may produce hydrogen gas and cause bulging on long storage.

Coagulation of the milk in the can may vary from a few flakes to a solid curd by species of *Bacillus* either mesophiles, such as *B. cereus*, *B. subtilis*, and *B. megaterium*; a facultative thermophile, *B. coagulans*; or an obligate thermophile, *B. calidolactis*. The extent of the curd in the milk depends to some extent on the amount of air in the can. Spoilage by the thermophiles should cause no trouble if the milk is cooled promptly and kept cool, but it can cause trouble in the tropics.

Bitterness usually results from proteolysis by species of *Bacillus* and less commonly by species of *Clostridium*. Some of the latter may cause putrefaction in rare instances.

Spoilage resulting from leakage by the presence of non spore formers, result in gas and swelling caused by coliform bacteria or yeasts, coagulation by streptococci, or bitterness caused by cocci.

Sweetened Condensed Milk

Sweetened condensed milk has been subjected to a fairly high temperature (71.1 to 100°C) during fore warming and to a milder heat treatment (48.9 to 54.4°C) during condensing, so that the yeasts, molds, and most of the vegetative cells of bacteria are destroyed. In addition there is a high concentration of sugar, about 55 to 60 percent of total sugar (lactose plus added

sugar). Also, the can is evacuated and sealed. Spoilage is due to primarily by the organisms that have entered after the heat treatments, especially if air is present.

The chief types of spoilage are

- (1) Gas formation by sucrose-fermenting yeasts or, more rarely, by coliform bacteria.
- (2) Thickening caused by micrococci, which probably produce rennet like enzymes.
- (3) "Buttons," which are mold colonies growing on the milk surface. The size of these buttons is determined by the amount of air in the can. Species of *Aspergillus*, e.g., *A. repens*, and *Penicillium*

FROZEN DESSERTS

Frozen desserts include ice cream, ice milk, frozen custards, sherbets, and ices. The ingredients may be various combinations of milk, cream, evaporated milk, condensed milk, dried milk, coloring materials, flavors, fruits, nuts, sweetening agents, eggs and egg products, and stabilizers. Any of these may contribute microorganisms to the product and affect the quality of the dessert as judged by its bacterial content or its content of specific kinds of bacteria such as the coliforms. The desserts are not ordinarily subject to spoilage, however, as long as they are kept frozen and if it is held at temperatures above freezing for a considerable time, souring occurs by acid-forming bacteria.

BUTTER

Many of the defects of butter originate in the cream from which it is made, especially when the cream has been held for several days on the farm before collection by the creamery. During this time lactic acid bacteria, gas formers, and other spoilage organisms may grow and be followed by molds, e.g., *Geotrichum candidum*. Lactose-fermenting yeasts, which are present only occasionally, may develop high gas pressures in the can of cream.

Flavour Defects:

Undesirable flavors may come from the cream, which may receive such flavors from the feed of the cow, absorb them from the atmosphere, or develop them during microbial growth. Feeds such as onions, garlic, French weed, peppergrass, and poor silage contribute off-flavors to the cream. Volatile products that may be absorbed from the air are odors from the barn and from the chemicals used there, e.g., kerosene, gasoline, fly sprays, disinfectants, etc. Growth of microorganisms in the cream and in the milk from which it is separated may result in any of the following bad flavors:

1. Cheesiness, caused by lactobacilli
2. Rancidity, resulting from lipolytic bacteria and molds and perhaps by lipase in the cream

3. Barny flavor, produced by species of *Enterobacter*
4. Malty flavor, produced by *Streptococcus lactis* var. *maltigenes*.
5. Yeasty flavor, produced by yeasts
6. Musty flavors, caused by molds and actinomycetes
7. Metallic flavors, caused by dissolved metals in highly acid cream
8. Flat flavor, resulting from the destruction of diacetyl by bacteria like some of the *Pseudomonas* species
- 9 Highly acid flavor, when the cream has excessive acidity.
10. "Unclean" flavor, caused by coliform bacteria

Unsatisfactory processing methods may cause a cooked flavor from over pasteurization of the cream or a "neutralizer" flavor if too much of the neutralizing compound is used, if it is unevenly distributed in the cream, or if pasteurization takes place before a balance is reached.

Like cream, butter readily absorbs volatile materials from the air. Microorganisms in the butter can cause the following defects:

1. **Surface taint**, also called "rabbito" and "putridity," which is blamed on *Pseudomonas putrefaciens*, introduced usually by the wash water, churns, or equipment. It is worse in unsalted or low-salt butter. The "sweaty-feet" odor is due chiefly to isovaleric acid.
2. **Fishiness**, caused by *Aeromonas hydrophila*.
3. **Esterlike flavors**, resulting from the action of *P. fragi*.
4. **Skunk like flavors**, caused by *P. mephitica*.
5. **Roquefort like** flavors, produced by molds.

Chemically produced flavors include

- (1) Rancidity produced by lipase in the cream,
- (2) Tallowiness from oxidations of unsaturated fats catalyzed by copper and bacterial enzymes and favored by a low pH, low-temperature pasteurization, salt, air, and ozone, and
- (3) Fishiness, where trimethylamine is produced from lecithin. This defect is favored by high acidity, salt, overworking of the butter, and the presence of copper.

Color Defects

Some color defects not caused by microorganisms are mottling because of improper working, a pink color caused by the sulfur dioxide refrigerant on the butter color, surface darkening resulting from the loss of water from surface layers, and bleaching that accompanies tallowiness.

Discolorations chiefly at the surface, may be caused by molds, yeasts, or bacteria that come from churns, wrappers, liners, circles, tubs, the air, or the cream if it is unpasteurized. Colored growths of molds result in the smudged or *Alternaria* type of discoloration, with dark, smoky, or (rarely) greenish areas where *Alternaria* or *Cladosporium* species have grown, or small black spots of *Stemphylium*. *Penicillium* produces green coloration, *Phoma* or *Alternaria* molds produce brown areas, and *Geotrichum* (syn. *Oospora*) species produce orange or yellow spots. *Fusarium culmorum* can cause bright reddish-pink areas. Yeasts sometimes grow in pink colonies. *Pseudomonas nigrifaciens* causes the reddish brown "black smudge" in mildly salted butter.

LECTURE-20

Microbiology of fruits and vegetables, contamination, preservation of vegetables, asepsis, chilling, freezing, drying, preservatives CA storage, MA storage. Spoilage of fruits and vegetables.

Contamination:

Fruits and vegetables are gathered into boxes, lugs, baskets, trucks during harvesting and they are subject to contamination with spoilage organisms. From each other and from the containers unless these have been adequately sanitized.

Mechanical damage during transportation to market may increase susceptibility to decay and growth of microorganisms may take place. Precooling of the product and refrigeration during transportation will slow such growth.

Soaking, washing by agitation tend to distribute spoilage organisms from damaged to whole foods. Recirculated or reused water is likely to add organisms and the washing process may moisten surfaces enough to permit growth of organisms during a holding period. Washing with detergent or germicidal solutions will reduce numbers on the foods.

Sorting spoiled fruits or vegetables or trimming spoiled parts removes microorganisms, but additional handling may result in mechanical damage. The spraying gives a fresh appearance to the vegetables and delays decomposition but also adds organisms Ex: Psychrotrophs

In the processing plant, the fruits & vegetables are subjected to further contamination. Adequate washing at the plant causes a reduction in numbers of micro organisms peeling by steam, hot water or lye and blanching reduces micro organisms.

Sweating of products during handling increases microbial numbers. Equipments or instruments may add microorganisms during processing.

Wooden surfaces are difficult to clean and sanitize and therefore are especially likely to be sources of contamination.

Hot water blanching reduces total numbers of organisms on the food, but may cause the building of spores of thermophilic bacteria, causing the spoilage of canned foods Ex: Flat sour spores in peas. Buildup of populations of micro organisms on equipment as the result of microbial growth may greatly influence the amount of contamination.

Inclusion of decayed parts of fruits increases the numbers of micro organisms in fruit juices. Added ingredients such as sugar and starch may add spoilage organisms, especially spores of thermophilic bacteria.

Preservation of vegetables:

Asepsis:

Limited amount of contamination of vegetables takes place between harvesting and processing or consumption i.e., gross contamination can be avoided. Boxes, lugs, baskets and other containers should be practically free of the growth of micro organisms and some will need cleaning and sanitation.

Vegetables if they contact with spoilage vegetables. Heat resistant spores of spoilage bacteria may be present.

Ex: Flat sour bacteria, putrefactive anaerobes (*Clostridium thermosaccharolyticum*)

Removal of micro organisms:

Washing of vegetables removes most contaminants on the surface but leaves natural microbial surface flora.

Wash water is of good bacteriological quality, it should not add organisms.

Chlorinated water is used for washing.

Part of the mold growth on strawberries can be removed by washing with a nonionic detergent solution.

Use of heat:

Vegetables to be dried or frozen and some to be canned are scalded or blanched to inactivate their enzymes. 1000 to 10,000 fold are decreased.

Use of Low temperatures:

Root crops, potatoes, cabbage, celery can be preserved for a limited time by cellar storage.

Chilling:

Most vegetables are cooled and kept at chilling temperature before preservation. Chilling is accomplished by use of cold water, vacuum cooling used for lettuce.

Hydrocooling: Cooling before normal cold storage is done immediately after harvesting by use of a cold water spray, a practice referred to as hydrocooling. Freshening of leafy vegetables by a water spray will cool the products and help in preservation. Potatoes turn sweet at temperatures below 2.2 – 4.4°C. Sweet potatoes and onions are subjected to special curing treatments before storage.

Freezing:

Freezing process reduces the number of organisms. *Micrococcus* are predominant on thawing vegetables such as sweet corn and peas.

Achromobacter and *Enterobacter* spp are also commonly present. *Lactobacillus* are also common on peas under such conditions.

One species of *Micrococcus* may grow at first followed by another species later. At higher temperatures, sps of *Flavobacterium* also may multiply.

During freezing most vegetables wilt and become limp and during storage frozen vegetables may undergo color changes. When thawed vegetables are held at room temperature for any considerable period, there is a chance that food poisoning bacteria may grow and produce toxin.

Enterotoxin forming staphylococci found in frozen corn.

Drying:

Dried vegetables and vegetable products are used in dried soups. Dried spices and condiments are used as flavoring materials.

Explosive puffing: Many vegetables can be dried by a process called explosive puffing. Usually small pieces of the diced, partially dehydrated vegetables are placed in a closed rotating chamber. Heat is applied and the chamber is pressurized to a pre determined level, then the pressure is released instantaneously. It results in additional loss of water, but more important, a porous network of capillaries is formed in the product. The increased porosity simplifies further drying and imports good reconstituting ability.

Drying by heat destroys yeasts and most bacteria, but spores of bacteria and molds usually survive. Dried vegetables are sulfured to preserve a light color, their microbial content is reduced. Spores of the bacteria and molds, Micrococci and Microbacteria are resistant to dessication and will survive better than other micro organisms.

Use of preservatives:

Rutabagas and turnips sometimes are paraffined to lengthen their keeping time.

Zinc carbonate has been reported to eliminate most mold growth on lettuce, beets, and spinach.

Biphenyl vapors will control *Fusarium* on potatoes.

Controlled atmosphere of CO₂ or ozone about chilled vegetables have little practical use.

Added preservative:

Sodium chloride is the only added chemical preservative in common use. The amount added to vegetables may vary from the 2.25 – 2.5% (Ex: Sauerkraut).

Lower concentrations of salt permit an acid fermentation by bacteria to take place, as the percentage of salt is increased, the rate of acid production becomes slower until a level of salt is reached that will permit no growth or production of acid.

Vegetables that are high in protein, such as green peas and lima beans, as well as some cauliflower are preserved by the addition of enough salt to prevent any fermentation. i.e., 70 - 80°C salometer (18.6 to 21.2% salt) to saturation 26.5% salt (100° salometers).

Use of sulfites as salad fresheners become popularity to prevent enzymatic browning of lettuce, cole slaw and other salad items. Sulfite residues in foods may be associated with asthmatic attacks.

Developed preservatives:

At room temperatures, acid fermentation is normal for shredded, chopped or crushed vegetables containing sugar by lactic acid bacteria.

Undesirable flavors and changes may be due to the growth of coliform bacteria, bacilli, anaerobes, proteolytic bacteria and others. Addition of salt reduces the growth of undesirable microorganisms salt draws the juice from the vegetables and bring about better distribution of the lactic acid bacteria.

Amount of sugar in the vegetable affects the acidity that can be produced, while the amount of salt and the temperature determine the rate of acid production and the kinds of bacteria involved in it.

String beans and corn involves the addition of low salt at the start, amounts are increased as the fermentation continues, and finally enough salt added to prevent further growth of bacteria.

Preservation by irradiation:

Treatment with gamma rays to inactivate micro organisms causing decay, followed by storage has resulted in discoloration, softening or other deterioration of most vegetables.

Irradiation used successfully to delay sprouting of potatoes, onions and garlic and to kill insect reproduction on some vegetables.

Preservation of fruits and fruit products:

Asepsis:

Care should be taken to avoid contamination during harvesting, processing and from containers. Before harvest, fruits are usually exposed to insecticides and fungicides may have their flora altered by such treatments.

Removal of Micro organisms:

Washing – Removes dirt, micro organisms and poisonous sprays.

Washing may be with water, detergent solutions, bactericidal solutions such as chlorinated water.

Trimming also removes micro organisms.

Clear fruit juices may be sterilized by filtration.

Use of Heat:

Fruits seldom are blanched before other processing because blanching causes excessive physical damage.

On the basis of their pH fruits are divided into

Acid foods	High acid foods
Tomatoes, pears and pine apples	Berries

Steam pressure sterilizer is not required for most fruits, since heating at about 100°C is sufficient and can be accomplished by flowing steam or boiling water. More acid the fruit, less heat required for its preservation.

Use of low temperatures:

Apples can be preserved for a limited time in common or cellar storage.

Chilling:

Each fruit has its own optimal temperature and relative humidity for chilling storage. Fruits are treated with hypochlorites, sodium bicarbonate, borax, propionates, biphenyl, O-phenyl phenols, sulfur dioxide, thiourea, thiabendazole, dibromo tetrachloro ethane before or during storage.

Fruits also have been enclosed in wrappers treated with chemicals Ex: Sulfite paper on grapes, iodine paper on grapes and tomatoes, borax paper on oranges. Waxed wraps, paraffin oil, paraffin, waxes and mineral oil have been applied for mechanical protection.

CA Storage:

(CA) It implies the altering of various gases from normal atmospheric concentrations; usually this is done by increasing the CO₂ concentration and decreasing the O₂ concentration.

MA storage (modified atmosphere storage) used to describe CA conditions which are not accurately maintained or conditions where the air is initially replaced with gas but no further measures are taken to keep the gas atmosphere constant.

Gas storage means CA or MA storage.

Under certain circumstances only one gas is used. Ex: packaging a product in 100% N₂.

Optimal concentrations of CO₂ and O₂ vary with kind and variety of fruits.

CO₂ storage employed with apples, pears, bananas, citrus fruits, plums, peaches, grapes and other fruits.

Ozone in concentrations of 2 to 3 ppm in the atmospheres has been reported to double the storage time of loosely packed small fresh fruits, such as straw berries, raspberries, currants and grapes and delicate varieties of apples.

Ethylene in the atmosphere is used to hasten ripening or produce a desired color change and it is not considered preservative, although a combination of this gas and activated hydrocarbons has been suggested for the preservation of fruits.

Freezing:

During preparation of fruits for freezing, undesirable changes may take place such as darkening, deterioration in flavor and spoilage by micro organisms especially molds.

Washing the fruit removes most of the soil micro organisms some fruits are frozen in large drums.

Yeasts like *Saccharomyces*, *Cryptococcus*, molds like *Aspergillus*, *Penicillium*, *Mucor*, *Rhizopus*, *Botrytis*, *Fusarium*, *Alternaria* have been reported to be the predominant organisms in frozen fruits.

Yeasts are most likely to grow during slow thawing. Considerably lower than in frozen vegetables. Large numbers of mold hyphae may be indicative of the freezing of inferior fruit that included rotten parts.

Added sugar or increased concentration of the juice has a protective effect against killing.

Coliform bacteria mostly *Enterobacter aerogenes* form part of the natural flora of fruits.

Tests are conducted for the fecal coliforms like or *Escherichia coli* *Streptococcus faecalis*.

Drying:

Number of microorganisms in dried fruits are comparatively low and that spores of bacteria and molds are likely to be the most numerous. Alkali treatment, sulfuring, blanching and pasteurization reduce numbers of micro organisms.

Use of preservatives:

Chemicals applied to the fruits chiefly as dips or spray or impregnated in wrappers for the fruits among substances that have been applied to the outer surfaces of fruits are waxes, hypochlorites, biphenyl and alkaline sodium O- phenylphenate. Wrappers for fruits have impregnated with a variety of chemicals include iodine, sulfite, biphenyl, O-phenylphenol plus hexamine and others.

As a gas or fog about the fruit, CO₂, ozone and ethylene plus chlorinated hydrocarbons have been tried. Sulfur dioxide and sodium benzoate are preservatives that have been added directly to fruits or fruit products. Green olives are the only fruits which are

preserved on a commercial scale with assistance from an acid fermentation. Lactic acid fermentation takes place in the fermented green tomatoes and Rumanian preserved apples.ss

Spoilage

Deterioration of raw vegetables and fruits due to

1. Physical factors
2. Action of their own enzymes
3. Microbial action
4. Combinations of above
5. Mechanical damage due to
6. Action of animals, birds, insects.
7. Wounding
8. Bursting
9. Freezing
10. Dessication
11. Other mishandling

Plant pathogen infection may takes place and unfit for consumption. Improper environmental conditions during harvesting, transit, storage and marketing may favor spoilage. Plant enzymes continue their activity in raw plant foods. If O₂ is available, the plant cells will respire as long as they are alive and hydrolytic enzymes can continue their action after death of the cell.

General types of microbial spoilage:

The most commonly occurring types of spoilage are as follows:

- 1. Bacterial soft rot:** Caused by *Erwinia caratovora*. These are fermenters of pectins. *Pseudomonas marginalis* and *Clostridium*, *Bacillus* sps have also been isolated from these rots. It results in a water soaked appearance, a soft mushy consistency and often a bad odor.
- 2. Gray mold rot:** *Botrytis cinerea*. Botrytis name derived from the gray mycelium of the mold. It is favored by high humidity and a warm temperature.
- 3. Rhizopus soft rot:** It is caused by sps of *Rhizopus* Ex: *Rhizopus stolonifer*. This rot is often soft and mushy. The cottony growth of the mold with small, black dots of sporangia often covers masses of the foods.
- 4. Anthrachose:** Usually caused by *Collectotrichum lindemuthianum*, *Collectotrichum coccodes*, defect is spotting of leaves and fruits or seed pods.

5. **Alternaria Rot:** Caused by *Alternaria tenuis*. Areas become greenish brown early in the growth of the mold and later turn to brown or black spots.
6. **Blue mold rot:** Caused by *Penicillium digitatum*. Bluish green color spores are produced.
7. **Downey mildew:** Caused by *Phytophthora brexia*.
8. **Watery soft rot:** Caused chiefly by *Sclerotinia sclerotiorum*. Found in mostly vegetables.
9. **Stem end rots:** Caused by *Diplodia*, *Alternaria*, *Phomopsis*, *Fusarium*.
10. **Black mold rot:** Caused by *Aspergillus niger*. Dark brown to black masses of spores of the mold termed “Smut” called by layperson.
11. **Black rot:** Caused by *Alternaria*, *Ceratostomella*, *Physalospora*.
12. **Pink mold rot:** Caused by pink spored *Trichothecium roseum*.
13. **Fusarium rots:** Caused by *Fusarium* sps.
14. **Green mold rot:** Caused by *Cladosporium*, *Trichoderma*.
15. **Brown rot:** Caused chiefly by *Sclerotinia* (*Monilinia fructicola*) sps.
16. **Sliminess or souring:** Caused by Saprophytic bacteria in piled, wet, heating vegetables.

Fungal spoilage of vegetables often results in water soaked, mushy areas. Fungal rots of fleshy fruits such as apples and peaches frequently shown brown or cream colored areas in which mold mycelia are growing in the tissue below the skin and aerial hyphae and spores may appear later. Some types of fungal spoilage appear as “dry rots” where the infected area is dry and hard and often discolored. Rots of juicy fruits may result in leakage. Bacterial soft rot is present in vegetables which are not very acid.

Spoilage of fruit and vegetable juices:

Molds can grow on the surface of acidic fruit juices if juices are exposed to air. High moisture content favors the faster growing of yeasts. The removal of solids from the juices by extraction and sieving raises the oxidation – reduction potential and favors the growth of yeasts. Most fruit juices are acid enough and have sufficient sugar to favor the growth of yeasts within the range of temperature that favours them from 15.6 to 35°C. The deficiency of B-vitamins discourages some bacteria.

In addition to the usual alcoholic fermentation, fruit juices may undergo other changes caused by microorganisms:

1. The lactic acid fermentation of sugars, mostly by heterofermentative lactic acid bacteria such as *Lactobacillus pastorianus*, *Lactobacillus brevis* and *Leuconostoc mesenteroides* in apple or pear juice and by homofermentative lactic acid bacteria such as *Lactobacillus arabinosus*, *Lactobacillus leichmanii* and *Microbacterium*

2. The fermentation of organic acids of the juice by lactic acid bacteria. E.x. *Lactobacillus psatorianus*, malic acid to lactic acid and succinic acid, quinic acid to dehydroshikimic acid, and citric acid to lactic and acetic acids
3. Slime production by *Leuconostoc mesenteroides*, *Lactobacillus brevis*, and *Lactobacillus plantarum* in apple juice and by *L. plantarum* and Streptococci in grape juice.

Vegetable juices contain sugars but less acid than fruit juices and have pH around 5.0 to 5.8. The accessory growth substances support growth of fastidious lactic acid bacteria by acid fermentation.

Concentrates of fruit and vegetable juices contain high sugar content. It favours the growth of yeasts like acid and sugar tolerant like *Leuconostoc* and *Lactobacillus* species.

LECTURE-21

Microbiology of cereal and cereal products contamination, preservation and spoilage of flours.

Contamination:

Exteriors of harvested grains retain some of the micro-organisms while growing and also adds contamination from soil, insects and other sources. Freshly harvested grains contain a few thousand to millions of bacteria per gram and several hundred thousand mold spores. Bacteria are mostly in the families Pseudomonadaceae, Micrococaceae, Lactobacillaceae and Bacillaceae.

Souring and washing the grains remove some of the microorganisms, but most of the microorganisms are removed with the outer portions of the grain during milling.

There is a possibility of contamination during other procedures such as blending and conditioning. Ex: Wheat flour contains spores of bacillus, coliform bacteria, *Achromabacter*, *Flavobacterium*, *Sarcina*, *Micrococcus*, *Alcaligenes* and *Serratia*. Fungi include *Aspergillus*, *Penicillium* and *Alternaria*, *Cladosporium*. Wheat flour from retail market contain 100 to 10^6 bacteria per gram and 20-30 *Bacillus* spores per gram, 50-100 mold spores per gram. Patent flours usually give lower counts. Cornmeal and flour contain 100 to several thousand bacteria and molds per gram. *Fusarium* and *Penicillium* are the dominant molds.

The surface of a freshly baked loaf of bread is practically free of viable micro organisms but they subject to contamination by mold spores from the air during cooling and before wrapping. During slicing, contamination may take place from micro organisms in the air, on the knives or on the wrapper.

The contamination of grains and cereal products with molds has become a significant concern because of the presence of aflatoxins. (Produced by *Aspergillus flavus* and *Aspergillus parasiticus*).

Preservation:

Most cereals have low a_w (water activity). Storage temperature of about 4.4 to 7.2°C is recommended for the dry products.

Asepsis:

Adequate cleaning and sanitization of equipment is essential for preservation. Improperly sanitized equipment may be source of rope bacteria, acid forming bacteria that causes sourness of doughs.

Bread, cakes and other baked goods that may be subject to spoilage by molds should be protected against contamination by mold should be protected against contamination by mold spores.

Use of Heat:

Bakery products which are unbaked, partially backed, fully baked. The complete baking process ordinarily destroys all bacteria cells, yeasts, and mold spores, but not spores of the rope forming or other bacteria.

Mold spores in prober cloths in bakeries can build up enough heat resistance to survive baking unbaked or partially baked products usually are kept on the retailers shelf for only a short period or kept cool during longer storage.

Ex: Boston brown bread and nut bread have been successfully canned.

Use of Low Temperatures:

Ordinary room temperature may be used by homemakers for short term storage of baked goods. Bakery goods can be stored in freezers. Unbaked or partially baked products, waffles, cheese cake, ice cream pie and fish, poultry and meat pies are usually frozen.

Bread and rolls can be stored successfully for months in the frozen condition.

Use of chemical preservatives:

Ammonia, propionic acid are used for preservation of bakery products. 2 % Ammonia and propionic acid (1%) reduce mold growth in high moisture corn. Sodium and calcium propionate, sodium diacetate and sorbates are used extensively. Acidification of the dough with acetic acid has been used to combat rope.

Use of Irradiation:

U.V rays have been used to destroy or reduce numbers of mold spores in dough and proof rooms, on the knives of slicing machines. Gamma and cathode rays have been applied experimentally for the preservation of baked goods. Low level irradiation can also be used to destroy insects in stored grains.

Spoilage:

Cereal grains and meals:

Cereal grains and meals are not processed to reduce their natural flora of microorganisms greatly, they are likely to contain molds, yeasts and bacteria. These microorganisms readily grow if moisture increases.

Unavailable to many organisms, these grains contain some sugar and available nitrogen compounds, minerals and accessory growth substances. Amylases will release more sugar and proteinase will yield more available nitrogenous foods if the grains are moistened.

Molds will grow on the surface, where air is available.

A wet mash of the grains or a mash of the meals will undergo an acid fermentation, chiefly by the lactic acid and coliform bacteria normally present on plant surfaces. This may be followed by an alcoholic fermentation by yeasts as soon as the acidity has increased enough to favour them. Finally molds will grow on the top surface, although acetic acid bacteria, if present may oxidize the alcohol to a acetic acid and inhibit the molds.

Ex: *Aspergillus*, *Penicillium*, *Mucor*, *Rhizopus* and *Fusarium*.

Flours:

Dry cleaning and washing grains and the milling and sifting of flour reduce the content of micro organisms.

White wheat flour usually bleached by an oxidizing agent such as oxide of nitrogen, chlorine, nitrosyl chloride, benzoyl peroxide.

Moisture content of flour less than 13% has been reported to prevent the growth of all micro organisms.

15% moisture permits growth of mold growth.

17% moisture permits growth of bacteria & molds.

Acid forming bacteria Ex: *Acetobacter* sps permits acetic acid fermentation.

Bacillus sps may grow, producing lactic acid gas, alcohol, acetoin and small amounts of esters and other aromatic compounds. It is the characteristic of most flour pastes to develop an odor of acetic acid and esters.

LECTURE-22

Microbiology of cereal and cereal products. Spoilage-Bread, Mold, Rope, Red bread, Chalky Bread.

Bread:

Acid fermentation by lactics and coliform bacteria that is normal in flour pastes or dough may be too extensive if too much time is permitted resulting that the dough and bread made from it may be too 'sour'. Excessive growth of proteolytic bacteria during this period may destroy some of the gas holding capacity so essential during the rising of the dough and produce a sticky dough.

Sticky doughs – due to the result of over mixing or gluten breakdown by reducing agents
Ex: glutathione. Production of undesirable flavours other than sourness.

Chief types of microbial spoilage of baked bread have been moldiness and ropiness, usually termed as mold and rope.

Mold:

Molds are most common and most important cause of the spoilage of bread and most bakery products. Temperatures attained in the baking procedure usually are high to kill mold spores in and on the loaf, so that molds must reach the outer surface or penetrate after baking. Contamination may come from air during cooling, handling, from wrappers and initiate growth in the crease of loaf and between the slices.

Chief molds involved in the spoilage of bread are

Bread mold – *Rhizopus stolonifer* or *Rhizopus nigricans* bread mold contains white cottony mycelium and black dots of sporangia.

Green spores are produced by *Penicillium expansum* *Penicillium stoloniferum*, *Aspergillus niger*.

Greenish or purplish-brown to black conidial heads and yellow pigment diffusing into the bread.

Monilia sitophila – produces pink conidia give a pink or reddish color to its growth. Species of *Mucor* or *Geotrichum* also contaminate the bread and produces spores.

Mold spoilage is favored by

1. Heavy contamination after baking due to air heavily laden with mold spores, a long cooling time, considerable air circulation or a contaminated slicing machine.
2. Slicing in that more air is introduced into the loaf.
3. Wrapping, especially if the bread is warm when wrapped.
4. Storage in a warm & humid place.

Little contamination occurs, if the relative humidity below 90%. Bread with 6% of milk solids retains moisture somewhat better than does milk free bread and hence there is less moisture between loaf and wrapper and hence less molding.

Various methods are employed to prevent moldiness of bread:

Prevention of contamination of bread with mold spores insofar as practicable. Air in the bread has low spores. Filtration and washing of air to the room and irradiation of the room and more especially the air by means of U.V. rays cut down contamination.

Prompt and adequate cooling of the loaves before wrapping to reduce condensation of moisture beneath the wrapper. U.V. irradiation of the surface of the loaf and of slicing knives.

Destruction of molds on the surface by electronic heating. Keeping the bread cool to slow mold growth or freezing and storage in the frozen condition to prevent growth entirely.

Incorporation of mycostatic material in the bread dough.

Ex: Sodium or calcium propionate @ 0.1-0.3%

Sodium acid – 0.1%

Sodium diacetate – 0.32%

Addition of vinegar or acetate to the dough or treatment of the exterior of the loaf with vinegar.

Rope:

Ropiness of bread is fairly common in home baked bread, especially during weather.

Ropiness is uncommon in commercially baked bread, because of the preventive measures now employed. Ropiness caused by mucoid variant of *Bacillus subtilis*, *Bacillus licheniformis*, (*B. mesentericus*), *B. panis* spores of these bacteria can withstand the temperature of the bread during baking, which does not exceed 100°C. Ropy condition due to capsulation of bacillus, together with hydrolysis of the flour proteins (gluten) by proteinases of the organism and starch by amylase.

Area of ropiness is yellow to brown in color and is soft and sticky to the touch. Sometimes slimy material can be drawn out into long threads, when the bread is broken and pulled apart. Unpleasant odor described as that of decomposed or overripe melons.

First odor is evident, then discoloration and finally softening of the crumb, with stickiness and stringiness.

Red bread:

Red bread or bloody bread is due to the occurrence of red color from the growth of pigmented bacteria *Serratia marcescens*. In ancient times, appearance of red color was considered miraculous.

Moist conditions favor growth. Molds such as *Monilia sitophila* may impart red color to bread. Red color in the crumb of dark bread has been caused by *Geotrichum aurantiacum* (syn, *Oidium aurantiacum*)

Chalky bread:

Chalky bread is due to white, chalk like spots. It is due to the yeast like fungi *Endomycopsis fibuligera* and *Trichosporon variable*.

Cakes & Bakery products:

Molds are the chief cause of spoilage of cakes and other bakery products. Topping or fillings are more prone to microbial spoilage than actual baked portion. Frostings due to high sugar content spoiled by molds or yeast upon storage. The deterioration of breads, cakes, pies is referred to as staling and it is due to mostly by physical changes during holding and not by micro organisms.

Pasta, Macaroni and Tapioca:

Pasta is egg based product containing flour, water and eggs, pasta is delivered and stored dry. Macaroni usually contains only flour, water and other nutrients. Swelling of moist macaroni has been reportedly caused by gas production by bacteria resembling *Enterobacter cloacae*. Purple streaks are produced by *Monilia*. Tapioca prepared from the root starch of cassava will spoiled by orange pigmented, starch hydrolyzing bacterium.

Breakfast cereals and other cereal snacks:

Breakfast cereals are flaked, puffed or extruded. In the initial steps of manufacturing of these products, there are high levels of moisture and therefore the possibility of microbial growth. Finished products contain low number of micro organisms.

Prepared doughs:

These products contains a yeast or a lactic acid bacteria inoculum. Microbial contamination depends on quality of ingredients used and sanitary practices employed.

LECTURE-23

Microbiology of Meat and Meat Products. Contamination, Preservation.

Contamination:

Inner flesh of meats is healthy and contains few or no micro organisms, but lymph nodes, bone marrow contain micro organisms.

Staphylococci, *Streptococci*, *Clostridium* and *Salmonella* have been isolated from the lymph nodes of red meat animals. Normal slaughtering practices would remove the lymph nodes from edible parts.

Mainly contamination comes from external sources during bleeding, handling and processing during bleeding, skinning and cutting the main sources of micro organisms are the exterior of the animal (hide, hooves and hair) and the intestinal tract. Recently approved “humane” methods of slaughter – mechanical, chemical and electrical have little effect on contamination, but each method is followed by sticking and bleeding causes contamination.

Exterior of the animal harbors large numbers a many kinds of micro organisms from soil, water, feed and manure as well as its natural surface flora and the intestinal contents.

Knives, clothes, air and hands as intermediate sources of contaminants. During handling of meat thereafter, contamination can come from carts, boxes or other containers, other contaminated meat, air and personnel.

Chilling storage adds psychrotrophic bacteria. Micro organisms isolated from fresh and refrigerated meat are

Bacteria: *Acinetobacter*, *Moraxella*, *Pseudomonas*, *Aeromonas*, *Alcaligenes* and *Micrococcus*

Molds: *Cladosporium*, *Geotrichum*, *Sporotrichum*, *Mucor* and *Thamnidium*.

Yeasts: *Candida*, *Torulopsis*, *Debaryomyces* and *Rhodotorula*.

Micro organisms isolated from processed and cured meats.

Bacteria: *Lactobacillus* and other lactic acid bacteria, *Acinetobacter*, *Bacillus*, *Micrococcus*, *Serratia* *Staphylococcus*.

Molds: *Aspergillus*, *Penicillium*, *Rhizopus* and *Thamnidium*.

Yeasts: *Debaryomyces*, *Torula*, *Torulopsis*, *Trichosporon* and *Candida*.

Yeasts are mostly asporogenous ones. Many of the bacteria grow at chilling temperatures. There is a possibility of the contamination of meat and meat products with human pathogens, especially those of the intestinal type.

Preservation of Meat and Meat Products

Asepsis:

Asepsis or keeping micro organisms away from meats as much as practicable during slaughtering and handling, permits easier preservation by any method. Storage time under chilling conditions may be lengthened, aging for tenderizing becomes less of a risk, curing and smoking methods are more certain and heating processes are more successful.

Water spraying of the animal before slaughter reduces no. of micro organisms on the animal from hair, skin, hide and foots.

Gross soil may be washed from surfaces, but the wash water may add organisms.

Use of hot water or sanitizer sprays under pressure is an effective way of decreasing the total numbers of bacteria on the surfaces of the carcasses.

Moldy or spoiled surface areas of large pieces of meat, especially hung or aged, meat may be trimmed off.

Films used to wrap meats keep out bacteria and affect the growth of those already have. These films differ considerably in their penetrability to water, oxygen and CO₂. Meats have been reported to have a shorter storage life in films less permeable to water. Fresh meats keep their red color better in an oxygen permeable film without evacuation.

With oxygen impermeable film, more CO₂ from bacteria is retained, resulting in a poorer color but favouring lactic acid bacteria, *Lactobacillaceae* and *Brochothrix thermosphacta*.

Cured meats preferably are packed in an oxygen tight film with evacuation. Evacuation helps restrict the growth of aerobes, especially molds, reduces the rate of growth of staphylococci and favors the growth of lactics but apparently does not favour the growth of *Clostridium botulinum* any more than plain over wrapping does.

Use of Heat:

Canning of meat is a very specialized technique. Most meat products are low acid foods that are good culture media for any surviving bacteria.

Commercially canned meats can be divided into two groups on the basis of the heat processing used.

Heat may be applied to meat products in other ways than canning. Treatment of meat surfaces with hot water to lengthen the keeping time has been suggested, although this may lessen nutrients and damage color. Cooking wieners at the packing plant by steam or hot water reduces the numbers of micro organism and helps preservation. Heat applied during the smoking of meats and meat products helps reduce microbial numbers.

Precooking or tenderizing of hams reduces bacterial numbers. Such products should be refrigerated. They are spoiled by food borne pathogens, if they are held at room temperatures.

Group - 1	Group - 2
1. Meats that are heat processed in an attempt to make the can contents sterile or at least “commercially sterile” as for canned meats for shelf storage in retail stores.	1. Meats that are heated enough to kill part of the spoilage organisms but be kept refrigerated to prevent spoilage. Canned hams and loaves of luncheon meats are so handled.
2. Shelf stable canned meats	2. Non shelf stable or keep refrigerated canned meats.
3. Processing temperature for shelf stable canned cured meats is 98°C.	3. Processing temperature is 65°C.
4. Size of the container is usually less than 1 lb.	4. Size of the container usually up to 22 lb.

Use of Low Temperatures:

Chilling:

Modern packing house methods involve chilling meat promptly and rapidly to temperatures near freezing and chilling storage at only slightly above the freezing point. Chilling temperatures vary from -1.4 to 2.2°C.

Time limit for chilling storage of beef is 30 days. Pork, lamb and mutton 1 to 2 weeks. Relative humidity usually is lowered with an increase in storage temperature.

Storage time can be lengthened by storage of meats in an atmosphere containing added CO₂, ozone or the temperature and relative humidity can be raised without shortening storage time. Increasing amounts of CO₂ in the atmosphere inhibit micro organisms but also hasten the formation of met myoglobin and hence the loss of “Bloom” or natural color. Storage life of meat has been doubled by such gas storage. CO₂ Concentration 10 – 30% for most meats and 100 % for Bacon.

Storage time also increased by the presence of 2.5 – 3 ppm ozone in the atmosphere. Storage up to 60 days at 2.2°C and 92% relative humidity without development of molds or slime. Ozone is an active oxidizing agent, and it gives an oxidized or tallowy flavour to fats. Microorganisms that give trouble in the chilling storage of meats are the psychrotrophic bacteria,

chiefly are *Pseudomonas*, *Acinetobacter*, *Moraxella*, *Alcaligenes*, *Micro coccus*, *Lacto bacillus*, *Streptococcus*, *Leuconostoc*, *Pediococcus*, *Flavobacterium*, *Proteus* and yeasts & molds.

Freezing:

Freezing often used to preserve meats during shipment over long distances. Large pieces of meat e.g., halves and quarters are sharp frozen, while hamburger and smaller, fancier cuts may be quick frozen in wrapped packages. The preservation of frozen meats is increasingly effective as the storage temperature drops from -12.2 towards -28.9°C .

Freezing process kills about half the bacteria and numbers decrease slowly during storage. The low temperature bacteria that grow on meat during chilling are *Pseudomonas* sps, *Acinetobacter*, *Moraxella*, *Alcaligenes*, *Micrococcus*, *Lactobacillus*, *Flavobacterium* and *Proteus* can resume growth during the thawing of meat if this is done slowly.

Use of Irradiation:

Irradiation with ultraviolet rays has been used in chilling storage to lengthen the keeping time. It has been employed chiefly on large, hung pieces of meat in plant storage rooms. Irradiation used in rapid aging of meats that are “hung” at higher than the usual chilling temperatures to reduce the growth of micro organisms especially molds on the surface.

Ordinary aging	U.V rays aging
80 – 90% RH. 3.3°C, air movement of 10-30 fpm. Several weeks.	18°C R.H – 85 – 90%. 2-3 days.

Gamma irradiation of meats still is limited. 20 – 70 kilo grams may cause undesirable changes in color and flavour may appear.

Preservation by Drying:

Drying meats for preservation has been practiced for centuries. Jerky or sun dried strips of beef was a standard food of American pioneers. Dried beef made mostly from cured, smoked beef hams, growth of micro organisms may takes place between processing and develop in the “pickle” during curing, but numbers of organisms are reduced by the smoking and drying process. Older methods of drying meats are usually combined with salting and smoking. During World war II pieces of freshly cooked beef and pork were dried by heat.

Another method of drying pork involves a short nitrate – nitrite cure before drying and the additional of lecithin as an antioxidant and stabilizer drying may be by vaccum, in trays or by other methods. Final product keeps without refrigeration. Freeze drying of meats is on the

increase, with greater success with processed products such as meat patties, and meat balls and stew than with fresh meats.

Use of Preservatives: Preservation of meat by heavy salting is an old method and leads to inferior product. Salting is combined with curing and smoking to get good quality product.

Curing:

Curing of meats is done for beef, pork, either ground meat or certain cuts such as hams, butts, jowls, sides, loins and bellies of hogs and the hams, brisket and leg muscles of beef. Ordinarily curing of meats is for the purpose of preservation of meats without refrigeration, but most cured meats in present days have other ingredients added and is refrigerated. Many of these are smoked and hence dried to some extent. Curing agents are NaCl, sugar, sodium nitrite and vinegar. NaCl, sugar and NaNO_2 are commonly used.

NaCl (sodium chloride or common salt):

1. Used primarily as a preservative and flavouring agent.
2. Cover pickle used for immersing the meat, may contain about 15% of salt, where as pumping pickle injected into the meat contain 24% sat.
3. Its primary purpose is to lower a_w (water activity).

Sugar:

1. It adds flavour and serves as an energy source for nitrate reducing bacteria in the curing solution or pickle.
2. Generally sucrose is used chiefly. Sometimes glucose is used or added if short term cure is employed or no sugar may be added.

Sodium nitrate:

1. It is indirectly a colour fixative and it is bacteriostatic in acid solution especially against anaerobes.
2. Nitrite can be formed by bacterial reduction during the long cure.
3. Sodium nitrite is the source of nitric oxide, which is the real color fixative and has some bacteriostatic effects in solution.

Sodium chloride have most preservative effect with some bacteriostatic effect from the nitrite and little effect from the nitrate.

Salts, sugar and meat protein combine to lower the water activity (a_w) of cure meats.

Ex: Hams – 0.95 to 9%

Nitrite:

1. Plays a role in the color of meats
2. Purplish red color of meats is due to blood haemoglobin and muscle myoglobin; oxymyoglobin which are bright red.
3. Under acid and reducing conditions in the presence of nitrite, the red nitric oxide myoglobin and nitroso haemoglobin are produced from myoglobin and haemoglobin.

Acid condition is produced by the meat itself, the reduced condition by the bacteria, and the nitric oxide for the reaction by reduction of the nitrite.

Four methods for introducing curing agents into meat:

1. The dry cure in which dry ingredients are rubbed into meat.
Ex: curing of Belly bacon
2. Pickle cure: Meats are immersed in a solution of the ingredients.
3. Injection cure: Concentrated solution of the ingredients is injected by needle into the arteries and veins of the meat via an artery or into muscular tissue in various parts of the meat. Ex: Pork hams.
4. Direct addition methods: Curing agents are added directly to finely ground meats. Ex: Sausage.

Curing temperature especially with a pickling, usually 2.2 – 3.3°C. Older methods of curing in the pickle require several months, but the newer “quick cure” in which the pickling solution is pumped into the meat greatly shortens that time. Meats are smoked after curing to improve preservation. Corned beef are not smoked but must be refrigerated.

Some types of sausage Ex: Thuringer, cervelat, Lebanon, Bologna, Salamis, dry & semidry sausages, undergo an acid fermentation mainly lactic acid kind during curing. This fermentation prevents undesirable fermentations, but also adds a desired tangy flavour. They contain mixed lactic flora, but the use of pure cultures such as *Pediococcus cerevisiae* have more control of the desired fermentation and shortens the fermentation time.

Vinegar is added to the pickling solution for preservation of pickled pigs feet, pickled spiced beef, and souses.

Pig's feet are cured in a solution of salt sodium nitrate and sodium nitrite, cooked and then held in a brine of salt and vinegar. Then they are packed into jars and covered with afresh salt – vinegar brine and the jar is sealed. Unless the acidity is unduly low, the product will not spoil.

Microbiology of meat curing Brines:

Micro organisms in curing brines and on immersed meats in vary with the initial condition of the meat and the method of curing employed. Red surface colonies of Halophilic bacteria develops on dried salted meat.

In modern American short methods of curing meats (Ex: Ham), bacteria in the brine have little to do with the changes that occur in the meat. These micro organisms are killed by smoking. Such brine solution contain lactic acid bacteria, except at the surface, where micrococci and yeasts may develop. Lactics are chiefly lacto bacilli and pediococci.

In olden methods of curing, especially *micrococci* functioned in reducing nitrate to nitrite, thus fixing the red color in the meat. Curing bacon involves immersion of concentrated brines for longer periods. These brines build up micrococci, a mixture of cocci and gram +ve and gram negative rods that form tiny colonies on agar media. These bacteria are halo tolerant to halophilic and reduces nitrates to nitrites. Hog bellies are treated with the dry curing mixture and compressed in boxes hence growth of salt tolerant, nitrate reducing psychrotrophs is permitted.

Beef curing brines may contain micrococci, Lacto bacilli, Streptococci, *Achromobacter*, vibrios and perhaps pediococci.

Sps of *Micrococcus* are active in many of the pickling solutions and have been found especially in those of high salt concentration used in British and Canadian Bacons.

Smoking:

Smoking has two main purposes 1. add desired flavours; 2. to help in preservation. Preservative substances added to the meat, together with the action of the heat during smoking. These substances inhibits microbial growth during storage. Older methods of curing and smoking, where high salt concentrations are present. Ex: Hams, dried beef are stored without refrigeration.

Newer methods give a perishable product which spoils and requires refrigeration.
Ex: Pre cooked or tenderized hams and sausages contain high moisture.

Spices:

Spices and condiments added to meat products such as meat loaves and sausages. They are not enough added for preservation. Ex: Bologna, Polish, Frankfurter and other sausages owe their keeping quality to the combined effect of spicing, curing, smoking (drying), cooking and refrigerating.

Antibiotics:

Permitted use of antibiotics in meats is in practice.

Fish – Chlortetracycline 7 ppm in ice.

Antibiotics increases shelf life at chilling or higher temperatures.

Mostly recommended antibiotics are chlortetracycline, oxytetracycline, nisin, chloramphenicol.

Antibiotics may be applied to meats in various ways. Antibiotic may be fed to the animal over a long period.

1. It may be fed more intensively for a short period before slaughter.
2. It may be infused into the carcass or into parts of it.
3. It may be applied to the surface of pieces of meat or mixed with comminuted meat.
4. Feeding an antibiotic brings about a selection of micro organisms in the animals intestinal tract, presumably reducing the numbers of spoilage bacteria.

LECTURE-24

Spoilage of meat and meat products. Invasion of tissues by microorganisms and growth of microorganisms in meat.

Raw meat may be spoiled by

1. Its own enzymes
2. By microbial action
3. Fat may be oxidized chemically.

Moderate amount of autolysis is desired in the tenderizing of beef and game by hanging or aging but not for other meats.

Autolytic changes are Proteolytic action on muscle and connective tissues and slight hydrolysis of fats. Defect caused by excessive autolysis has been called “souring”

General principles underlying meat spoilage:

1. Invasion of tissues by micro organisms: Upon death of the animal, invasion of the tissues by contaminating micro organisms takes place. Factors that influence that invasion include the following.

a) The load in the gut of the animal: Greater the load, greater is the invasion of tissues. For the reason starvation for 24 hr before slaughter has been recommended.

b) The physiological condition of the animal immediately before slaughter: If the animal is excited, feverish or fatigue, bacteria are more likely to enter the tissue. Therefore encouraging the spread of bacteria. Chemical changes may take place more readily due to better bacterial growth because of a higher and more rapid denaturation of proteins. Glycogen is used in fatigue, P^H will not drop from 7.2 to about 5.7, as it would normally.

c) The method of killing and Bleeding: Better and more sanitary the bleeding, better the keeping quality of the meat. More greening was found in pork and bacon from electrically stunned animals than from those killed with CO_2 .

d) The rate of cooling: Rapid cooling will reduce the rate of invasion of the tissues by micro organisms. Micro organisms are spread in the meat through the blood and lymph vessels and connective tissue interspaces and in ground meat by grinding.

Growth of Micro organisms in meat: Meat is an ideal culture medium for many organisms because it is high in moisture, rich in nitrogenous foods of various degrees of complexity and plentifully supplied with minerals and accessory growth factors.

Meat usually has fermentable carbohydrate (glycogen) and has favourable pH for most micro organisms.

Factors that influences the growth of micro organisms and kind of spoilage are as follows:

1. The kind and amount of contamination with micro organisms and the spread of these organisms in the meat.

Ex: Meat contaminated with high no. of Psychrotrophs would spoil at chilling temperatures.

2. The physical properties of the meat:

Amount of exposed surface of the flesh has considerable influence on the rate of spoilage because the greatest load of organisms present from the air. Aerobes will grow. Fat may protect some surfaces but fat itself subject to spoilage chiefly by enzymes and chemical.

Grinding meat greatly increases the surface and encourages microbial growth and it releases moisture and distributes bacteria throughout the meat.

Skin also protects the meat.

3. Chemical properties of the meat: Moisture content is important in determining the growth and kind of micro organism. If the surface is dry, no growth is permitted. A little moist allows mold growth; still moisture encourages yeasts and very moist encourages bacterial growth. Low content or absence of fermentable carbohydrate and high protein content tend to favour the non fermenting types of organisms.

P^H of raw meat is 5.7 to 7.2 depending on the amount of glycogen present.

4. Availability of oxygen: Aerobic conditions at the surface of meat are favourable to molds, yeasts and aerobic bacteria.

Within the solid pieces of meat, conditions are anaerobic. Oxygen may diffuse slowly into ground meat and slowly raise the O – R potential unless the casing or packaging material is impervious to oxygen. True putrefaction is favored by anaerobic conditions.

5. Temperature: Meat should be stored at chilling temperatures. Molds, yeasts and psychrotrophic bacteria grow slowly and produce characteristic defects.

True putrefaction is rare at low temperatures and commonly occurs at room temperatures.

At chilling temperatures, psychrophiles are favored and proteolysis caused by a dominating species of bacterium, followed by utilization of peptides and amino acids by secondary species.

At ordinary atmospheric temperatures, mesophiles would grow, such as coliform bacteria and sps of *Bacillus* and *Clostridium*, with the production of moderate amounts of acid from the limited amounts of carbohydrates present.

LECTURE-25

General types of spoilage of meats. Spoilage under anaerobic conditions, spoilage of different kinds of meats.

General types of spoilage of meats

Spoilage under aerobic conditions:

1. Surface slime: Caused by the species of *Pseudomonas*, *Acinetobacter*, *Moraxella*, *Alcaligenes*, *Streptococcus*, *Leuconostoc*, *Bacillus* and *Micrococcus*.

Some sps of *Lactobacillus* can produce slime.

Temperature and the availability of moisture influence the kind of micro organisms causing surface slime.

At chilling temperatures, high moisture will favour the *Pseudomonas* – *Alcaligenes* group; whereas less moisture favours *Micrococci*, yeasts (Ex: Frankfurters). Still less moisture – molds may grow at room temperatures. *Micrococci* and other *mesophiles* compete well with the *Pseudomonads* and related bacteria.

2. Changes in color of meat pigments:

Red colour of meat called as “bloom”. This bloom may be changed to shades of green, brown or gray as a result of the production of oxidising compounds Ex: Peroxides, Hydrogen sulfides, Species of *Lacto bacillus* (mostly hetero fermentative) and *Leuconostoc* causes greening of sausage.

3. Changes in Fats:

Oxidation of unsaturated fats in meats takes place in air and catalyzed by light and copper. Lipolytic bacteria may cause some lipolysis and accelerate the oxidation of fats. Butter fat becomes tallowy on oxidation and rancid on hydrolysis. Most animal fats develop oxidative rancidity when oxidized, with off odors due to aldehydes and acids.

Hydrolysis adds flavours of the released fatty acids rancidity caused by lipolytic sps of *Pseudomonas* and *Achromobacter* or by yeasts.

4. Phosphorescence:

Caused by phosphorescent or luminous bacteria.

Ex: *Photobacterium* spp. Growing on the surface of the meat.

5. Various surface colors due to pigmented bacteria:

Red spot caused by *Serratia marcescens*.

Blue colour caused by *Pseudomonas syncyanea*

Yellow pigments produced by the sps of *Micrococcus* or *Flavobacterium*.

Greenish blue to brownish black spots on stored beef caused by *Chromobacterium lividum*. Purple “stamping ink” discoloration of surface fat caused by yellow pigmented cocci and rods. When the fat becomes rancid and peroxides appear, the yellow color changes to a greenish shade and later become purplish to blue.

6. Off odors and off tastes:

“Taints” or undesirable odors and tastes that appear in meat as a result of the growth of bacteria on the surface often are evident before other signs of spoilage.

“Souring” is the term applied to almost any defect that gives a sour odor that may be due to volatile acids. Ex: Formic, Acetic, butyric and propionic acid or even growth of yeasts.

“Cold storage flavor” or taint is an indefinite term for a stale flavour.

Actinomycetes may cause musty or earthy flavour.

Yeasts may grow under aerobic conditions on the meats: Yeasts may grow under aerobic conditions on the meats and causes sliminess, lipolysis, off odors and tastes, discolorations (white, cream, pink or brown. Due to pigments in yeasts)

Aerobic growth of molds may cause the following:

- 1. Stickiness:** Incipient growth of molds makes the surface of the meat sticky to the touch.
- 2. Whiskers:** When meat is stored at temperatures, a limited amount of mycelial growth may take place without sporulation. White fuzzy growth can be caused by a number of molds including *Thamnidium chaetocladioides*, *Thamnidium elegans*, *Mucor mucedo*, *Mucor lusitanicus*, *Mucor racemosus*, *Rhizopus* and others.

Controlled growth of a special strain of *Thamnidium* has been recommended for improvement in flavour during aging of beef.

- 3. Black spot:** Usually caused by *Cladosporium herbarum*. But other molds with dark pigments may be responsible.

- 4. White spot:** Caused by *Sporotrichum carnis*. *Geotrichum* also causes white spot.

- 5. Green patches:** Caused by *Penicillium expansum*, *Penicillium asperulum*, *Penicillium oxalicum*.

- 6. Decomposition of Fats:** Many molds have lipases and hence cause hydrolysis of fats. Molds also help in the oxidation of fats.

- 7. Off odour and off tastes:** Molds give a musty flavour to meat in the vicinity of their growth. Sometimes this defect is given a name called “thamnidium taint”.

Spots of surface spoilage by yeasts and molds usually are localized to a great extent and can be trimmed off without harm to rest of the meat.

Extensive bacterial growth over the surface may bring fairly deep penetration. Then facultative bacteria may grow inward slowly.

Spoilage under Anaerobic conditions:

Facultative and anaerobic bacteria are able to grow with in the meat under anaerobic conditions and cause spoilage.

1. Souring: Souring implies a sour odor and perhaps taste. Caused by formic, acetic, butyric, propionic and higher fatty acids or other organic acids such as lactic or succinic.

Souring can result from

- (a) Action of meats own enzymes during aging or ripening.
- (b) Anaerobic production of fatty acids or lactic acid by bacterial action.
- (c) Proteolysis without putrefaction caused by facultative or anaerobic bacteria and sometimes called “Stinking sour fermentation”.

Acid and gas formation accompany the action of the “butyric” *Clostridium* sps and the coliform bacteria on carbohydrates.

Vacuum packed meats, especially those in gastight wrappers, commonly support the growth of lactic acid bacteria.

2. Putrefaction:

True putrefaction is the anaerobic decomposition of protein with the production of foul smelling compounds such as H₂S, Mercaptans, Indole, Skatole, Ammonia and Amines.

Putrefaction usually caused by sps of *Clostridium*.

Some other facultative bacteria responsible for putrefaction are *Pseudomonas Putrefaciens*, *P. putrificum*, *P. Putida* *Alcaligenes putrefaciens*, *A. Putrificum*, *A. Putida*.

Some species of *Proteus* are putrefactive. Trimethyl amine in fish and isovalenic acid in butter are described as putrid odors. Gas formation accompanies putrefaction by clostridia, and the gases are H₂ and CO₂.

3. Taint: Taint is a word applied to off-taste or off odor. The term “bone taint” of meats refers to either souring or putrefaction next to the bones, especially in hams.

True putrefiers require temperature above those of refrigerator. Meat held at 0°C – Molds, yeasts, bacteria will grow. They produce sliminess, discoloration and spots.

Spoilage of Different kinds of meats:

Spoilage of Fresh meats: Extended refrigeration may have the growth of *Pseudomonas*, *Acinetobacter*, *Moraxella* and causes spoilage of fresh meat.

Lactic acid bacteria chiefly of the genera *Lactobacillus*, *Leuconostoc*, *Streptococcus*, *Brevibacterium* and *Pediococcus* are present in most meats, fresh or cured and can grow even at refrigerator temperatures; their limited growth does not spoil the quality of the product. Lactic acid fermentation is favourable in certain types of sausages such as salami, Lebanon and thuringer. Lactic acid bacteria responsible for 3 types of spoilage.

1. Slime formation at the surface or within especially in the presence of sucrose.
2. Production of a green discoloration
3. Souring, when excessive amounts of lactic and other acids have been produced.

Fresh beef:

Fresh beef undergoes the changes in color mentioned.

1. Changes in the haemoglobin and myoglobin i.e, loss of bloom and the production of reddish brown met haemoglobin and metmyoglobin and the green gray brown oxidation pigments by action of oxygen and micro organisms.
2. White, green, yellow and greenish blue to brown black spots and purple discolorations due to pigmented micro organisms.
3. Phosphorescence
4. Spots due to various bacteria, yeasts and molds.

Beef also subject to sliminess on the surfaces due to bacteria, yeasts, stickiness due to molds, whiskers resulting from mycelial growth of molds, souring and putrefaction by bacteria. Pseudomonads usually predominate in beef held at 10°C or lower but at 15°C or above micrococci and pseudomonads grown in about equal numbers.

Hamburger:

Hamburger held at room temperatures usually putrefies, but at temperatures near freezing it acquires a stale, sour odour. Sourness at low temperature caused by *Pseudomonas*, *Acinetobacter* and *Moraxella* with help from *lactic acid bacteria*.

Alcaligenes, *Micrococcus* and *Flavobacterium* sps may grow in some samples.

Hamburger held at higher temperatures contain the micro organisms like *Bacillus*, *Clostridium*, *Escherichia coli*, *Enterobacter*, *Proteus*, *Pseudomonas*, *Alcaligenes*, *Lactobacillus*, *Leuconostoc*, *Streptococcus*, *Micrococcus* and *Sarcina*.

Fresh pork sausage:

Fresh sausage is made mostly of ground fresh pork to which salt and spices have been added. Pork sausage is a perishable food that must be preserved by refrigeration.

Souring the most common type of spoilage at temperature of 0 – 11°C. Souring due to the growth and acid production by lactobacilli and leuconostocs.

Souring at higher temperature also causes by the growth of *Microbacterium* and *Micrococcus*. Encased pork sausages especially “little pig” are subject to slime formation on the outside of the casing on long storage. Colored spots are due to mold growth. *Alternaria* cause small dark spots.

Spoilage of cured meats:

Cured meats are pork, some cuts of beef. Sodium nitrite lactic acid bacteria in sausages like *thuringer* & *Essex* and leads to *lactic acid fermentation*. Curing salts make meats more favourable to growth of *gram positive* bacteria. Yeasts and molds than to gram –ve.

Dried Beef or Beef Hams:

Beef hams are made spongy by *Bacillus* sps.

Sour – by variety of bacteria

Red – due to *Halobacterium salinarum* or red *Bacillus* sps.

Blue – *Pseudomonas syncyanea*, *Penicillium Spinulosum* (purplish), *Rhodotomela* sps.

As the relative humidity increases and the product absorbs water, there is a decrease in shelf life. Gas in jars of chipped dried beef has been attributed to denitrifying aerobic organisms that resembles *Pseudomonas fluorescens*. Gases are oxides of nitrogen.

Bacillus sps have been known to produce CO₂ in the jars.

Sausage:

Encased sausages, spoilage micro organisms may grow on the outside of the casing, between the casing and meat or in the interior. Growth of the organisms can take place on the outside of the casings only if sufficient moisture is available.

Ex: *Micrococcus* and yeasts can form slimy layer, often occurs on frank further that becomes moist when removal from refrigeration.

With less moisture molds produce fuzziness and discoloration. CO₂ produced by heterofermentative lactic acid bacteria, may swell packages of wieners or breakfast sausages when they are packaged in gastight flexible film.

Growth between the casing and the meat favoured by accumulation of moisture there during cooking if the casing is penetrable to water. When two casings are employed, inner casing may be wetted before the outer casing is applied, trapping water between them. Slime produced by acid producing micrococci.

Acid forming Micrococci (*Micrococcus candidus*) may grow in liver sausage and bologna. *Bacillus* sps may grow in liver sausage.

Psychrotrophic leuconostocs and lacto bacilli cause a souring in *Lebanon*, *Thuringer*, *Essex* sausage.

Fading of red color of sausage to chalky gray has due to oxygen and light and may be hastened by bacteria. Chilling rings are due to oxidation, production of organic acids or reducing substances by bacteria, excessive water and under cooking.

Greening of sausage is due to the production of peroxides (Ex: H_2O_2) by heterofermentative sps of *Lactobacillus* and *Leuconostoc* or other *catalase negative* bacteria. H_2S also may be involved. Greening is favored by slightly acid pH and by the presence of small amounts of oxygen. Greening occurs within 12 -36 hrs after the sausage has been processed even under refrigeration.

Green cores in large sausage Ex: Big Bologna, develop usually after 4 or more days of holding and within 1 – 12 hr after slicing. Greening of a cut surface indicates contamination with and growth of salt tolerant, peroxide forming bacteria (probably lactics) which can grow at low temperatures. Surface sliminess often accompanies the greening.

Production of nitric oxide gas in sausage by nitrate reducing bacteria has been reported.

Bacon:

Streptococcus faecalis often present because of its salt tolerance and ability to grow at low temperatures. Surface flora of Bacon contains Micrococci and staphylococci.

Molds are the chief spoilage organisms on the cured bacon, especially on the sliced, packaged bacon when stored in home refrigerator.

Most trouble is encountered in late summer and early fall with sps of *Aspergillus*, *Alternaria*, *Monilia*, *Oidium*, *Fusarium*, *Mucor*, *Rhizopus*, *Botrytis* and *Penicillium*. Sliced bacon may be deteriorated by oxidizing and lipolytic bacteria on long storage.

Oxidising and sulphide forming bacteria produces poor color in the flesh part of bacon.

Chromogenic bacteria may cause discoloured areas. Yellowish brown discolorations, showing the presence of tyrosine is due to the growth of proteolytic bacteria.

In the manufacturing of Wiltshire bacon, the sides of the hog are cured in concentrated brine for 6 – 8 days at 3.3 – 4.5°C. It permits the growth of only psychrotrophic, salt tolerant bacteria. Unopened, packaged sliced bacon is spoiled mostly by lactobacilli, but micrococci and fecal streptococci may grow, especially if the wrapper is somewhat permeable to O_2 . Opened bacon may be spoiled by molds.

Bacon prepared with 40 – 80 ppm sodium nitrite, 0.7% sucrose and a *Lacto bacillus plantarum* had a greater *antibotulinal* effect than bacon made with 120 ppm sodium nitrite only.

Ham:

Souring of hams due to non odors proteolysis to genuine putrefaction with its very obnoxious odors of mercaptans, hydrogen sulphide, amines, indole etc. Caused by a large variety of psychrotrophic, salt tolerant bacteria. Souring caused by *Alcaligenes*, *Bacillus*, *Pseudomonas*, *Lactobacillus*, *Proteus*, *Serratia*, *Bacterium*, *Micrococcus*, *Clostridium*.

Hydrogen sulphide producing streptobacilli that cause flesh souring of ham.

Types of sourings are classified according to their location as sours are classified according to their location as sours of shank or tibial marrows, body or meat, aitchbone, stiffl joint, body bone or femur marrow and buff. Puffers or gassy hams are not encountered commonly but occur occasionally. Quick cure or common method for curing hams reduces the souring. In this method curing solution is pumped into the ham.

Tenderized hams are really precooked and are given a mild cure. Such hams are perishable and should be protected from contamination and refrigerated during storage to prevent their deterioration by micro organisms. Improperly handled tenderized hams may be spoiled by *E. coli*, *Proteus*, *Staphylococcus aureus*.

Refrigerated packaged meats:

Packaging films, permitting good penetration of O₂ and hence CO₂ favor the more aerobic bacteria, such as pseudomonas, Acinetobacter and Moraxella and their production of flavours, slime and even putrefaction. Films with poor gas penetration encourage lactic acid bacteria, especially when combined with vacuum packing. These bacteria in time cause souring, slime, and atypical flavours.

Curing solutions (or) pickles:

Spoilage of the pickle or curing solution for ham and other cured meats is likely in the presence of available sugar and a pH well above 6.0. Multiuse brines generally spoiled by putrefactive bacteria. Ex: *Vibrio*, *Alcaligenes* or *Spirillum*.

Souring can be caused by *Lactobacillus* and *Micrococcus* and slime by *Leuconostoc* or *Micrococcus lipolytics*.

Turbid and ropy vinegar about pickled pigs feet or sausages are caused chiefly by lactic acid bacteria from the meats, although yeasts may be responsible for cloudiness.

Black spots on pickled pig's feet caused by hydrogen sulfide producing bacteria and gas in vacuum packed pickles may from hetero fermentative lactic acid bacteria or yeasts.

LECTURE-26

Microbiology of fish and other sea foods. Contamination, preservation, spoilage

Sea foods include fresh, frozen, dried, pickled and salted fish as well as various shell fish and fresh water.

Contamination:

Microbial flora of living fish depends on the water in which they live.

Slime on the outer surface of fish contain *Pseudomonas*, *Acinetobacter*, *Moraxella*, *Alcaligenes*, *Micrococcus*, *Flavobacterium*, *Corynebacterium*, *Sarcina*, *Serratia*, *Vibrio* and *Bacillus*.

Bacteria on fish from northern waters are mostly psychrophilic, whereas fish from tropical water plus species of *Aeromonas*, *Lactobacillus*, *Brevibacterium*, *Alcaligenes* and *Streptococcus*.

Intestines of fish from both sources are found bacteria of the genera *Alcaligenes*, *Pseudomonas*, *Flavobacterium*, *Vibrio* and *Bacillus*, *Clostridium* and *Escherichia*.

Boats, boxes, bins, fish houses and fishers soon become heavily contaminated with these bacteria.

Oyster and other shell fish contain *Alcaligenes*, *Flavobacterium*, *Moraxella*, *Acinetobacter*. These fish thrush water bodies and pick up soil and micro organisms.

Number of micro organisms on the skin of fish can be influenced by the method of catching.

Trawling fish nets along the bottom for long periods results in exposure of the fish to high bacterial counts in the sediment. Fish cakes and fish sticks may have contamination from potatoes, spices and flavorings etc.

Preservation:

Of all the flesh foods, fish is the most susceptible to autolysis, oxidation and hydrolysis of fats and microbial spoilage. Evisceration should be done promptly to stop active digestive enzymes in the gut.

Rigormortis is especially important in the preservation of fish, for it retards postmortem autolysis and bacterial decomposition. Any procedure that lengthens rigor mortis lengthens keeping time.

Aseptic methods to reduce the contamination of seafood are difficult to apply. Gross contamination can be removed before processing by general cleaning and sanitization of boats, decks, holds, bins.

Use of Heat:

Live crabs are cooked in retorts at temperatures up to 121°C to facilitate removing the meat from the shell. Hand picking and hand packing of the meat is common.

Processing times and temperatures for canned crabmeat range from 85.6 to 87.2°C for 92 – 150 min. These processes are considered pasteurization and cans are preserved by refrigeration.

Most canned sea foods, however are heat processed so that they are sterile or atleast “commercially sterile”.

Sea foods are low acid foods. They have slow rate of heat penetration and hence are difficult to heat process.

Canning practices carried out in accordance with the FAO code of practice and minimize health hazards arising from canned sea foods.

Use of Low Temperature:**Chilling:**

Fish flesh autolyzes and the fats become oxidized at temperatures above freezing rapidly at summer temperatures and more slowly as the temperature is dropped toward freezing.

Preservation by chilling is temporary. Necessity of chilling is required from the catching area to processing plant. Small fish are more perishable than whole dressed fish autolyze more slowly than whole fish but are spoiled more readily by bacteria.

If outside temperatures are warm and distance of transportation are great, it becomes necessary to chill the fish and are stored in crushed ice or mechanical refrigeration.

Incorporation of preservatives in the ice used for chilling fish is common practice.

Freezing:

In earlier days ice with added salt was employed. Now mechanical refrigeration includes sharp freezing and the fish were “glazed” i.e., a layer of ice was frozen around the outside.

Whole fish – usually sharp frozen in air or in salt brine.

Quick freezing is applied to wrapped fillets or steaks.

Quick frozen fish may thaw to like their original condition than fish frozen more slowly.

During storage the fats of frozen fish are subject to hydrolysis and oxidation.

Fatty fish deteriorate more rapidly than lean ones. Cooked shrimp are frozen.

Other sea foods preserved by freezing include scallops, clams, oysters, spiny lobster tails and cooked crab and lobster meat.

Freezing kills some but not all of the micro organisms present and growth will take place after thawing if time permits.

Fish carry a flora of psychrotrophic bacteria, most of which survive freezing and are ready to grow on thawing.

Ex: *Pseudomonas*, *Acinetobacter*, *Moraxella*, *Alcaligenes* and *Flavobacterium* sps.

Spores of Type E *Clostridium botulinum* will survive freezing and storage and may grow and produce toxin when temperatures reach 3.3°C or above.

Frozen raw sea foods contain few enterococci, coliforms or staphylococci.

Use of Irradiation:

Gamma or cathode irradiation applied to some kinds of fish.

U.V rays has been tried but not in practice.

Preservation by Drying:

Dry salting of fish or immersion in brine constitutes a method of drying. In this, moisture is removed or tied up.

Oxidation of fish oils is not retarded and may cause deterioration.

Salt cod is prepared by a combination of salting and air drying. Flesh is removed from bones and skin. Smoking of fish results in drying of the fish.

Use of preservatives:

Salting or marination of fish by dry salt or in brine is effective not only because of the drying effect mentioned but also because of the effect of sodium chloride.

Fish may be drysalted so as to contain 4-5% salt. Salts contributes halophiles which may discolor the fish. Ex: Red colour due to *Serratia salinarum*.

Micrococcus sps may grow on the fish and decrease in *flavobacterium*, *Alcaligenes*, *pseudomonas* and others.

Curing of fish may be “mild” i.e., with light salting or may be in heavy brine or with solid salt and followed by smoking.

Benzoic acid and benzoated are moderately successful.

Sodium and potassium nitrites and nitrates lengthens the keeping time. These chemicals are permitted in some countries only.

Sorbic acid delays spoilage of smoked or salted fish.

Boric acid has been used in Europe with some improvement in the keeping quality, but use of boric acid is illegal in united stated.

Other chemicals used as preservatives but very limited are formaldehyde, hypochlorited, hydrogen peroxide, sulfur oxide, undecylenic acid, capric acid, p – oxybenzoic acid and chloroform.

Antibiotics also have been used as dip or in ice. Chlortetracycline and oxytetracycline are best and their use is permitted. Penicillin, streptomycin and subtilin are poor

Storage of fish in an atmosphere containing increased levels of CO₂ has been found to lengthen the keeping time. Normal spoilage flora is replaced with Lactobacilli and other the product “sours” when it begins to spoil.

Pickling of fish means that salting or acidification with vinegar, wine or sour cream.

Herring is treated in various ways. Salted, spiced and acidified.

Formerly fish was smoked primarily for its preservation and the smoking was heavy, but now canning, chilling, freezing are available to lengthen keeping time.

Much of the smoking of fish is primarily for flavor and hence is light.

Fish to be smoked usually are eviscerated and decapitated but may be in the round, split or cut into pieces.

Commonly, salting, light or heavy, precedes smoking and serves not only to flavor the fish but also to improve its keeping quality by reducing the moisture content.

Drying may be aided by air currents.

Smoking done at comparatively at low temperatures 26.7 to 37.8°C or at high temperatures 63°C to 92°C. High temperature smoking results in partial cooking of the fish.

Microbiology of fish Brines:

Number of bacteria in fish curing brines vary with the concentration of salt, temperature of the brine, kind and amount of contamination from the fish introduced and duration of use of the brine. Number of micro organisms range from 10,000 to 10million bacteria per milliliter. (10^4 to 10^6 / ml).

Salt concentration usually between 18% and saturation but may be lower, especially after fish are introduced.

Higher the temperature of the brine, the more salt necessary to prevent its spoilage.

Generally contamination includes pseudomonas, Acinetobacter, moraxella, Alcaligenes and Flavobacterium.

Ice introduces cocci and coryne bacterium.

Continued use of brine, increases number of micro organisms because addition of successive lots of fish.

Salt tolerant bacteria such as micrococci is also present.

As the brine ages, there is a decrease in numbers and an increase chiefly in coryne bacteria in low salt brines and in micrococci in high salt brines.

Preservatives incorporated in ice:

Germicidal ices are prepared by adding a chemical preservative to water before freezing.

Eutectic ice	Non eutectic ice
When the added chemical is uniformly distributed throughout. Ex: NaCl	When the added chemical not uniformly distributed throughout. Ex: Sodium benzoate Non eutectic ice is finely crushed for use on fish so as to get the chemical evenly sprad in it.

Many chemicals have been tried for incorporation in ice for icing fish.j

Ex: Hypochlorites, chloramines, benzoic acid, benzoated, colloidal silver, H₂O₂, O₃, sodium nitrite, sulfonamides, antibiotics, propionates, levulinic acid and many others.

American and canadian governments now permit the incorporation of the tetracyclines up to 7ppm in ice to be used by fishers to presserve fish on trowlers and during transportation.

Antioxidants:

Fats and oils of fatly fishes (Ex: Herring, mackerel, mullet and salmon) are comosed of unsaturated fatty acids and hence are subject to oxidative changes producing oxidative rancidity and sometimes undesirable alterations in colour.

To counteract these undesirable changes, antioxidants may be applied as dips, coatings, glazes or gases.

Good results have been reported with nordihydroguaiaretic acid, ethyl gallate, ascorbic acid and other compounds and with storage in carbon dioxide.

Spoilage

Fish spoiled by autolysis, oxidation or bacterial activity or combination of these.

Fish flesh is more perishable than meat because autolysis by the fish enzymes and because of the less acid reaction of fish flesh that favors microbial growth. Unsaturated fish oils are more susuptible to oxidative deterioration than animal fats.

Rigor mortis hastened by struggling of the fish, lack of oxygen, warm temperature.

Rigor mortis delayed by low p^H adequate cooling of the fish.

Spoilage occurs or begins until after rigor mortis, when juices are released from the flesh results from the conversion of muscle glycogen to lactic acid.

LECTURE-27

Factors influencing kind and rate of spoilage, evidences of spoilage, bacteria causing spoilage.

Factors influencing kind and rate of spoilage:

1. The kind of fish:

Flat fish spoil more readily than round fish because they pass through rigor mortis more rapidly. But flat fish like halibut keeps longer because of the low P^H (5.5) of its flesh.

Certain fatty fish deteriorate rapidly because of the oxidation of the unsaturated of their oils. Fishes high in triethylamine oxide soon yield appreciable amounts of the “stale-fishy” trimethylamine.

2. Condition of the fish when caught:

Fish that are exhausted as the result of struggling, lack of oxygen and excessive handling spoil more rapidly than those brought in with less ado, probably because of the exhaustion of glycogen and hence smaller drop in p^H of the flesh.

“Feedy fish” i.e., those full of food when caught, are more perishable than those with an empty intestinal tract.

3. The kind and extent of contamination of the fish flesh with bacteria:

Contamination occurs from mud, water, handlers, exterior slime and intestinal content of the fish. They are supposed to enter the gills of the fish, from which they pass through the vascular system and thus invade the flesh. Contamination takes place in the net, fishing boat on the docks etc.

4. Temperature:

Chilling the fish is the most commonly used method for preventing or delaying bacteria growth and hence spoilage until the fish is used or otherwise processed. Cooling should be rapid as possible 0 to -1°C and this low temperature should be maintained. Obviously the warmer the temperature, the shorter the storage life of the fish still more effective in its preservation.

5. Use of an antibiotic ice or dip.

Evidences of spoilage:

The change is gradual from a fresh condition to staleness and then to inedibility it is difficult to determine or agree on the first appearance of spoilage.

Practical tests to determine the quality of fish has been sought for many years, but none has proved entirely satisfactory.

Trimethylamine test used on salt water fish.

Estimate of volatile acids or volatile bases or a test for pH, H_2S , ammonia etc. are used. Bacteriological tests are too slow to be useful.

Succession of external changes in a fish:

Bright characteristic colors of the fish fade, and dirty yellow or brown discoloration appear. The slime on the skin of the fish increases, especially at the flaps and gills. The eyes gradually sink and shrink, the pupil becoming cloudy and the cornea opaque.

The gills turn a light pink and finally grayish yellow color. Most marked is the softening of the flesh, so that it exudes juice when squeezed and becomes easily indented by the fingers.

The flesh is easily stripped from along the backbone, where a reddish brown discoloration develops toward the tail and is a result of the oxidation of hemoglobin.

Sequence of odors is evolved. First the normal, fresh, seaweedy odor then a sickly sweet one then a stale fishy odor, due to trimethyl amine, followed by ammonical and final putrid odors due to hydrogen sulfide (H_2S), indole and other malodorous compounds. Fatty fish may also show rancid odors. Cooking will bring out the odors more strongly.

Bacteria causing spoilage:

Bacteria involved in the spoilage of fish are natural flora of the external slime of fishes and their intestinal contents.

Predominant kinds of bacteria causing spoilage vary with the temperatures at which the fish are held, but at the chilling temperatures usually employed, sps of pseudomonas are most likely to predominate, with *Acinetobacter*, *Moraxella* and *Flavobacterium* sps. *Micrococcus* and *Bacillus* are less often appears.

Normal pseudomonads increase in numbers on chilled fish during holding, Achromobacters decrease and Flavobacteria increases temporarily and then decreases. Bacteria grow first on the surfaces and later penetrate the flesh.

Fish have a high content of non protein nitrogen and autolytic changes caused by their enzymes increase the supply of nitrogenous foods. (Ex: Amino acids and animals) and glucose for bacterial growth. From these compounds the bacteria make trimethylamine, ammonia, amines (Ex: Putrescine and cadaverine), lower fatty acids and aldehydes and eventually hydrogen and other sulfides, mercaptans and indole, which products are indicative of putrefaction.

A musty or muddy odor and taste of fish has been attributed to the growth of *Streptomyces* sps in the mud at the bottom of the body of water and absorption of the flavor by the fish.

Discolorations of the fish flesh may occur by *Pseudomonas fluorescens*, yellow micrococci and others.

Red or pink color produce from growth of *Sarcina*, *Micrococcus*, *Bacillus* or by molds or yeasts. Chocolate brown color occurs by asporogenous yeast. Pathogens parasitizing the fish may produce discolorations or lesions.

Spoilage of special kinds of fish and sea foods:

Salt fish are spoiled by salt tolerant or halophilic bacteria (*Serratia*, *Micrococcus*, *Bacillus*, *Alcaligenes*, *Pseudomonas* and others). These bacteria cause discolorations, a red color being common.

Molds are the chief spoilage organisms on smoked fish. Marinated (sour pickled) fish should present no spoilage problems unless the acid content is low enough to permit growth of lactic acid bacteria or the entrance of air permits mold growth.

Japanese fish subject to souring caused by volatile acid production by Bacilli or to putrefaction, despite the addition of nitrite and permitted preservatives.

In chilled shrimps, *Acinetobacter*, *Moraxella* and *Vibrio* are chief responsible for spoilage.

Crabmeat deteriorated by *Pseudomonas*, *Acinetobacter*, *Proteus* at higher temperatures.

Raw lobsters are spoiled by *pseudomonas*, *Alcaligenes* and *Flavobacterium*.

Crabs and oysters may contain sps of *Vibrio* including *Vibrio parahaemolyticus*.

Oysters remain in good conditions as long as they are kept alive in the shell at chilling temperature, but they decompose rapidly when they are dead, as in shucked oysters.

At temperatures near freezing *Pseudomonas*, *Acinetobacter* and *Moraxella* species are the most important spoilage bacteria but *Flavobacterium* and micrococcus sps also may grow. The spoilage is termed “souring” although the changes are chiefly proteolytic.

At higher temperatures the souring may be the result of the fermentation of the sugars by coliform bacteria, Streptococci, Lactobacilli and yeast to produce acids and a sour odor. Early growth of *Serratia*, *Pseudomonas*, *Proteus* and *Clostridium* may takes place. An uncommon type of spoilage by asporogenous yeast causes pink oysters.

LECTURE-28

Microbiology of Poultry and Eggs. Contamination, preservation, spoilage. Changes during storage. Changes not caused by microorganisms and changes caused by microorganisms.

Contamination:

The skin of live birds may contain numbers of bacteria averaging 1,500 per square centimeter. These numbers probably reflect the natural flora of the skin plus other organisms that could be derived from feet, feathers, and feces.

Contamination of the skin and the lining of the body cavity occurs during washing, plucking, and evisceration. Chickens are currently processed by a fully automated conveyor or track line with vacuum evisceration. Bacterial numbers vary considerably on the surface of chickens. This variation, however, is greater between birds than it is between different areas of the same bird. The types of organisms isolated depend on where samples are taken and on the stage of processing.

The giblets (gizzards, hearts and livers) are processed separately, and numbers and types of microorganisms present may differ from those of the carcass. *Salmonella*-positive birds is common and range from 0 to 50 percent. There is also a high incidence of *Campylobacter jejuni* in poultry processing plants and on the processed bird.

Preservation of Poultry Meat: As in the slaughter of animals for meat, the method of killing and bleeding the fowl has an important effect on the quality of the product.

If the trachea is left intact, it is referred to as an outside cut.

A kosher cut severs the trachea. When the birds are scalded, they may gasp, drawing scald water into the air sac. Apparently the kosher cut minimizes inhalation of scald water because the cut end of the trachea is drawn under the skin.

Dry-plucked birds are more resistant to decomposition than semi scalded or scalded ones because the skin is less likely to be broken but more pinfeathers are left. Most picking is by means of the semi scald method, in which the fowl is immersed in water at about 55 C for 2 min.

The contamination to birds is minimized by

- (1) The temperature of the scald water.
- (2) The low initial count on some birds.
- (3) A dilution effect of adding fresh water to the scald tank. Steam scalding of birds is more effective than hot water in reducing numbers of bacteria, including coliforms and salmonellae.

ASEPSIS:

The sanitation of the housing of the birds before killing has some influence on the numbers of microorganisms on the skin at dressing. Contamination of the lining of the body cavity of the bird can be prevented if the fowl is not eviscerated until sold in the retail market, but visceral taints may develop unless the birds are adequately refrigerated. The shackles holding the feet and head of the fowl may be a source of heavy contamination. Contamination can be reduced if equipment is adequately cleaned and sanitized at intervals.

Use of Heat:

Dressed chickens and other fowls may be canned, whole or dissected, in their own juices or in jelly. Heat processes are analogous to those for canned meats. The chicken or other fowl may be salted in weak brine before being packed into the glass jars or cans.

Use of low temperature: Most poultry is preserved by either chilling or freezing. Various commercial methods of submerging the birds in cold water, ice water, or ice slush are used. Variations include counter flow vat-type, counterflow tumbler-type, and oscillating vat-type chiller, agitated ice and water, and blasts of cold air or CO₂ or sprays of refrigerant or solid CO₂. The chill tanks can serve as a source of contamination if not properly controlled. A counter flow system would be better in preventing the buildup of microbial numbers, since fresh potable water added to the chiller would flow against the movement of the carcasses. Chlorine can be added to chill-tank water to reduce numbers of organisms.

1. Chilling: Chilling storage of poultry is for only a short period, usually less than a month. Birds to be stored longer should be frozen. Packing the dressed birds in ice has been used for short periods of holding and where mechanical refrigeration is not available.

2. Freezing: The storage temperature should be below -17.8° C and the relative humidity above 95 percent to reduce surface drying.

Most poultry is sharp-frozen at about - 29° C or less in circulating air or on a moving belt in a freezing tunnel. For quick freezing, a smaller package is necessary, usually a whole bird, a cut-up one, or a boned fowl, packed in a special watertight and almost airtight wrapper.

Although some of the bacteria are killed by freezing process and numbers decrease slowly during storage, enough remain to cause spoilage when the bird is thawed. The growth of bacteria can take place during picking, dressing, drawing, chilling, and also during the freezing process until the temperature of the bird drops below 0° C.

Low-temperature bacteria of the genera *Proteus* and *Alcaligenes*, as well as coliform bacteria, have been found in considerable numbers. Canning will destroy them, but quick freezing permits many to survive.

Use of Preservatives:

Feeding antibiotics to birds may lead to increased percentages of resistant microorganisms in the fecal matter and hence on the birds. Low levels of antibiotic may be deposited in the flesh. Low levels of antibiotic in the meat of treated birds are mostly destroyed by cooking. Soaking cut-up poultry in solutions of organic acids (acetic, adipic, succinic, etc.) at pH 2.5 lengthens shelf life.

Turkey sometimes is cured in a solution of salt, sugar, and sodium nitrate for several weeks at about 3.3°C, washed, dried, and then smoked. Usually a light smoking process is used, more for flavor than for preservation.

Recommended temperatures during smoking range from 43.3 to 60° C, and the time ranges from a few hours to several days.

Carbon dioxide Atmosphere: Increasing carbon dioxide concentrations (10 to 20 percent) in the atmosphere of stored chickens inhibits the growth of psychrotrophs. Dry ice packed with the carcasses may serve as the source of the carbon dioxide. The use of films of both high and low gas permeability in combination with a carbon dioxide atmosphere reduces the microbial count.

Use of Irradiation: Irradiation of poultry with cathode or gamma rays is a successful preservation method since the rays apparently produce less objectionable change in appearance and flavor than in some other foods. But to date the method has not been practiced commercially. Radiation doses of 1 to 10 kilograys would reduce the microbial flora and extend the product's refrigerated shelf life.

SPOILAGE:

The enzymes of the fowl contribute to the deterioration of the dressed bird. Bacteria are the chief cause of spoilage. Intestines are primary source of these organisms. Bacterial growth takes place on the surfaces, i.e., the skin, the lining of the body cavity, and any cut surfaces, and the decomposition products diffuse slowly into the meat. Eviscerated poultry held at 10⁰ C or below is spoiled mostly by *Pseudomonas* and to a lesser degree by yeasts, e.g., *Torulopsis* and *Rhodotorula*. Above 10⁰C, micrococci usually predominate, and there also is growth of *Alcaligenes* and *Flavobacterium*. In time the surface of the meat usually becomes slimy.

Small amounts (1 to 5 ppm) of iron in the wash water may favor bacterial growth on the surface and production of the fluorescent pigment pyoverdine by *Pseudomonads*. More iron will reduce pigmentation.

About 100 ppm of magnesium is optimal for pigment production by *P. fluorescens*.

Iced, cut-up poultry often develops a slime that is accompanied by an odor described as "tainted," "acid," "sour," or "dishraggy." This defect is caused chiefly by species of *Pseudomonas*, although *Alcaligenes* also may be concerned.

MICROBIOLOGY OF EGGS

Contamination:

Most freshly laid eggs are sterile, at least inside, but the shells soon become contaminated by fecal matter from the hen, by the cage or nest, by wash water if the eggs are washed, by handling, and perhaps by the material in which the eggs are packed. The total number of microorganisms per shell of a hen's egg has been reported to range from 10^2 to 10^7 with a mean of about 10^5 . The types of microorganisms recovered from the shell are diverse. *Salmonella* sps may be on the shell or in the egg as laid, build up during processing, and appear in significant numbers in frozen or dried eggs.

Preservation

The egg has various ways of protecting itself from microbial invasion. The shell and the thin surface layer of proteinaceous material known as the **cuticle or bloom** are the first line of defense and serve to retard entry. However, the shell is porous for gas exchange during embryonic development. The membranes inside the shell also tend to serve as a mechanical barrier.

Albumen is an inadequate growth medium for many microorganisms. The characteristics of albumen that hinder microbial growth include a pH of 9 to 10 that may be reached during storage. Low levels of simple nitrogenous compounds like apoprotein ties up riboflavin, avidin ties up biotin, antiproteolytic factor prevent bacterial proteinases from releasing nitrogenous compounds necessary for growth, conalbumin (ovotransferrin) chelates iron and lysozyme is an enzyme which degrades the cell walls of gram-positive bacteria.

Asepsis: Care should be taken to reduce the contamination of the outside of the shell by hen feces and dirt from the nests. When the eggs are broken for drying or freezing care is taken to discard those in which microbial growth has taken place. Reduce contamination from equipment by cleaning and sanitizing it.

Removal of microorganisms: Dirty eggs command a lower price than clean ones, various methods have been tried for the removal of soil.

Dry cleaning, as by sandblasting, removes dirt and also the bloom (mucin).

Washing with warm, plain water removes dirt, the bloom, and part of the microorganisms but encourages the penetration of bacteria into the egg through the pores in the shell.

Cleaning disinfecting the washing machines with 1 % hypochlorite solution reduces contamination with rot bacteria. The use of 1 to 3% acetic acid was effective in removing the flora but resulted in a reduction of shell thickness and egg quality. Lye, acids, formalin, hypochlorites, quaternary ammonium compounds, various detergents, and detergent-sanitizer combinations have been tried in washing solutions.

Use of heat: The heat coagulability of the egg white determines to a great extent the maximal heat treatment that can be given shell eggs.

Rotting was well controlled by heating at 60⁰C for 320 seconds. Heating in water was superior to heating in oil.

A thermostabilization method of dipping eggs into hot water reduces evaporation of moisture from the egg by a slight coagulation of the outermost part of the egg albumen.

Pasteurization is required for most egg products. Pasteurization is not done for salt yolk for use in salad dressings. This product is exempt if it contains at least 1.4 percent acetic acid (pH 4.1 or lower). Because of the heat coagulability of eggs, a stabilization process is required before pasteurization. This includes the addition of aluminum salts and adjustment of the pH.

Use of low temperatures:

Chilling

Most shell eggs are preserved by chilling. They are selected for storage on the basis of their general appearance and the result of **candling**.

Candling: To candle an egg, it is held and rotated in front of a light to examine it for defects such as cracks, rots, molds, blood, developing embryo, crusted or sided yolk, weak white, or large air cell.

The eggs should be cooled after production and held at a temperature and relative humidity that depend on the anticipated time of storage. The lower the relative humidity below 99.6 percent, the more rapidly the egg will lose moisture and hence weight and the larger the air cell will become. The higher the relative humidity, the more likely the microbial spoilage of the egg.

The higher the temperature above -1.67⁰ C, the more rapid the penetration of microorganisms into the egg. For commercial storage for 6 months or longer, a temperature of -1.7 to -0.55⁰ C

and a relative humidity of 70 to 80 percent are recommended. Special treatments given eggs can improve their keeping quality during chilling storage. Impregnation of the eggshell with a colorless and odorless mineral oil is a common method that keeps out moisture, slows desiccation and air penetration, retains carbon dioxide, and retards physical and chemical changes within the egg. Eggs are sprayed lightly with the oil, particularly eggs to be stored commercially for long periods, with little apparent effect on rates of microbial changes within the egg.

Freezing

The chief bacteriological problems in the freezing of egg "meats" or "pulp" are in connection with the selection and the preparation of the egg contents for freezing. The eggs first are selected by candling and then are washed mechanically with a final rinse in 200 to 500ppm chlorine (iodine is also effective) and then broken on automatic breaking machines. The use of automatic machine breaking demands strict control over quality of the whole eggs and inspection of broken eggs before mixing or "pooling" of large quantities.

The egg meats whole or separated, are filtered to remove pieces of shell and stringy material (chalazae), mixed or churned, standardized as to solids content, and frozen in 30- or 50-lb tin cans or other containers, usually by a sharp-freezing process. Yolks frozen separately and stored frozen form a jelly which does not become fluid on thawing. This difficulty is avoided by the incorporation of 5 percent or more of sugar, salt, or glycerol before freezing. The frozen eggs are stored at -17.8 to -20.5°C.

Frozen eggs may contain high numbers of bacteria, up to millions per gram from contamination of egg pulps, contamination from pieces of shell and from equipment in the breaking room, and growth before freezing.

Bacteria that spoil eggs stored at low temperatures, especially *Pseudomonas* organisms, are likely to be numerous, as well as bacteria of the genera *Alcaligenes*, *Proteus*, and *Flavobacterium*. Gram-positive cocci and rods and coliforms from the eggshell occur in smaller numbers, and possibly anaerobes and other bacteria as chance contaminants. Occasionally, salmonellae may be in eggs from infected hens or contamination.

Preservation by drying

Egg white requires some additional treatment before drying to retain its whipping properties. Removal of glucose, one of the reactants in the Browning or Maillard reaction, must be done prior to drying. Several methods of glucose removal have been suggested, including fermentation by group D streptococci, *Enterobacter aerogenes*, or *Saccharomyces cerevisiae* and an enzyme

process using glucose oxidase. In the latter glucose is converted into gluconic acid, with added hydrogen peroxide serving as the oxygen donor. A cold desugaring process using glucose oxidase at 10°C has the advantage of producing a product with a low bacterial count.

Most of the drying of eggs done by spray dryer, where the liquid is sprayed into a current of dry, heated air. Another method is the roller or drum process, in which the liquid egg is passed over a heated drum, with or without vacuum.

Air drying is accomplished by means of open pans, as used by the Chinese, or by the belt system, where the egg liquid is on a belt that passes through a heated tunnel (60 to 71.1°C). Spray drying or pan drying, combined with tunnel drying, is used for egg white. Formerly, eggs were dried to a moisture of about 5 percent, but the keeping quality of dried white or whole egg is improved as the moisture content is decreased toward 1 percent, and the trend is in that direction.

Dried eggs may contain few hundred microorganisms per gram up to over 100 million, depending on the eggs broken and the methods of handling employed.

A variety of organisms have been found in dried eggs, including micrococci, streptococci (enterococci), coliform bacteria, salmonellae species, spore-forming bacteria, and molds. Cocci and the gram-positive rods are likely to be present as a result of contamination from the shell during breaking or from handlers or equipment rather than from spoiled eggs. Salmonellae can come from infected hens; however, they are not usually present, and most buyers specify "salmonella-free" products.

In fact, during storage the numbers of organisms in dry egg will decrease, more rapidly at first and gradually later. Organisms resistant to desiccation, such as micrococci and spores of bacteria and molds, will make up an increasing percentage of the survivors as storage continues.

Use of preservatives

Preservatives may be used on the shells of eggs, in the atmosphere around them, or on wraps or containers for eggs. An enormous number of different substances have been applied to the surface of the shells of eggs or used as packing material about eggs to aid in their preservation. Some of these substances are used primarily to keep the shell dry and reduce penetration of oxygen into the egg and passage of carbon dioxide and moisture out; waxing, oiling the shells and otherwise sealing are examples.

Other materials inhibit the growth of microorganisms, and some are germicidal. Materials used for the dry packing of eggs in the home include salt, lime, sand, sawdust, and ashes.

Immersion in water glass containing solution of sodium silicate. It is a successful home method of preservation. The solution is inhibitory because of its alkalinity. Other inhibitory chemicals that have been tried are borates, permanganates, benzoates, salicylates, formates. The utilization of warm or hot solutions of hypochlorites, lye, acids, formalin, quaternary ammonium compounds and detergent sanitizer combinations. Sealing the shell with a solution of dimethylourea inhibits mold growth.

The only two gases that are added to the atmosphere about eggs to improve their keeping quality are carbon dioxide and ozone.

Use of Irradiation:

Salmonella in liquid, frozen, and dried eggs can be inactivated by means of ionizing radiation.

Spoilage of Eggs:

Defects in the fresh egg: Fresh eggs may exhibit cracks, leaks, loss of bloom or gloss, or stained or dirty spots on the exterior as well as "meat spots" (blood clots), general bloodiness, or translucent spots in the yolk when candled.

Changes during storage: The changes that take place in eggs while they are being held or stored may be divided into those due to non microbial causes and those resulting from the growth of microorganisms.

Changes Not Caused by Microorganisms:

1. Untreated eggs lose moisture during storage and hence lose weight. The amount of shrinkage is shown to the candler by the size of the air space or air cell at the blunt end of the egg, a large cell indicating much shrinkage.
2. The change in the physical state of the contents of the egg, as shown by candling or by breaking out the egg.
3. The white of the egg becomes thinner and more watery as the egg ages, and the yolk membrane becomes weaker.
4. When an old egg is broken onto a flat dish, the thinness of the white is more evident and the weakness of the yolk membrane permits the yolk to flatten out or even break. By contrast, a broken fresh egg shows a thick white and a yolk that stands up strongly in the form of a hemisphere.
5. During storage, the alkalinity of the white of the egg increases from a normal pH of about 7.6 to about 9.5. Any marked growth of the chick embryos in fertilized eggs also serves to condemn the eggs.

Changes Caused by Microorganisms

To cause spoilage of an undamaged shell egg, the causal organisms must do the following (1) contaminate the shell, (2) penetrate the pores of the shell to the shell membranes (usually the shell must be moist for this to occur), (3) grow through the shell membranes to reach the white (or to reach the yolk if it touches the membrane), (4) grow in the egg white, despite the previously mentioned unfavorable conditions there, to reach the yolk, where they can grow readily and complete spoilage of the egg.

In general, more spoilage of eggs is caused by bacteria than by molds. The types of bacterial spoilage, or "rots," of eggs go by different names.

Alford et al. (1950) list five groups of rots that are found in Australian eggs for export. Among the three chief ones are

1. **Green rots**, caused chiefly by *Pseudomonas fluorescens*, a bacterium that grows at 0°C; the rot is so named because of the bright-green color of the white during early stages of development. This stage is noted with difficulty in candling but shows up clearly when the egg is broken. Odor is lacking or is fruity or "sweetish." The contents of eggs so rotted fluoresce strongly under ultraviolet light.

2. **Colorless rots**, which may be caused by *Pseudomonas*, *Acinetobacter*, *Alcaligenes*, certain coliform bacteria, or other types of bacteria. These rots are detected readily by candling, for the yolk usually is involved, except in very early stages, and disintegrates or at least shows a white incrustation. The odor varies from a scarcely detectable one to fruity to "highly offensive."

3. **Black rots**, where the eggs are almost opaque to the candling lamp because the yolks become blackened and then break down to give the whole-egg contents a muddy-brown color. The odor is putrid, with hydrogen sulfide evident, and gas pressure may develop in the egg. Species of *Proteus* most commonly cause these rots, although some species of *Pseudomonas* and *Aeromonas* can cause black rots. *Proteus melanovogenes* causes an especially black coloration in the yolk and a dark color in the white.

The development of black rot and of red rot usually means that the egg has at some time been held at temperatures higher than those ordinarily used for storage.

Pink rots: Pink rots occur less often, and red rots are still more infrequent. Pink rots are caused by strains of *Pseudomonas*. They resemble the colorless rots, except for a pinkish precipitate on the yolk and a pink color in the white.

Red rots: Red rots caused by species of *Serratia*, are mild in odor and are not offensive.

Spoilage of Eggs by Fungi:

The spoilage of eggs by fungi goes through stages of mold growth that give the defects their names.

1. Pin spot molding: Very early mold growth is termed pin-spot molding because of the small, compact colonies of molds appearing on the shell and usually just inside it. The color of these pin spots varies with the kind of mold. *Penicillium* species cause yellow or blue or green spots inside the shell, *Cladosporium* species give dark-green or black spots, and species of *Sporotrichum* produce pink spots.

2. Superficial fungal spoilage: In storage atmospheres of high humidity a variety of molds may cause superficial fungal spoilage, first in the form of a fuzz or "whiskers" covering the shell and later as more luxuriant growth. When the egg are stored at near-freezing temperatures, the temperatures are high enough for slow mycelial growth of some molds but too low for sporulation, while other molds may produce asexual spores. Molds causing spoilage of eggs include species of *Penicillium*, *Cladosporium*, *Sporotrichum*, *Mucor*, *Thamniidium*, *Botrytis*, *Alternaria*, and other genera.

3. Fungal rotting: The final stage of spoilage by molds is fungal rotting, after the mycelium of the mold has grown through the pores or cracks in the egg. Jellying of the white may result, and colored rots may be produced, e.g., fungal red rot by *Sporotrichum* and a black color by *Cladosporium*, the cause of black spot of eggs as well as of other foods. The hyphae of the mold may weaken the yolk membrane enough to cause its rupture, after which the growth of the mold is stimulated greatly by the food released from the yolk.

4. Off-flavors: Off-flavours sometimes are developed in eggs. Mustiness may be caused by any of a number of bacteria, such as *Achromobacter perolens*, *Pseudomonas graveolens*, and *P. mucidolens*. The growth of *Streptomyces* on straw or elsewhere near the egg may produce musty or earthy flavors that are absorbed by the egg. Molds growing in the shell also give musty odors and tastes.

A hay odor is caused by *Enterobacter cloacae*.

Fishy flavors are produced by certain strains of *Escherichia coli*.

The "cabbage-water" flavor mentioned in connection with type II black rot of Haines may appear before rotting is obvious. Off-flavors, such as the "cold-storage taste", may be absorbed from packing materials.

LECTURE-29

Microbiology of sugar and sugar products. Sources of contamination, spoilage and Prevention

Sugar products include sucrose (cane & beet sugar), molasses, syrups, maple sap and sugar, honey and candy.

Contamination:

Sucrose:

Raw juice from sugarcane may be contaminated with micro organisms during processing. Mainly contamination occurs from the adhering soil particles to the sugarcane and include slime producers.

Ex: *Leuconostoc* & *Bacillus*, *Micrococcus*, *Flavobacterium*, *Alcaligenes*, *Xanthomonas*, *Pseudomonas*, *Erwinia*, *Enterobacter*.

Yeasts include – *Saccharomyces*, *Candida*, *Pichia*

Much contamination may come from debris or fine particles on the sides or joints of troughs at the plant.

Microbial growth in sugarcane juice leads to destruction of sugar or inversion of bacteria.

Extraction and clarification of the juice may kill yeasts and vegetative cells of bacteria.

Sedimentation, filtration, evaporation, crystallization, centrifugation reduces number of micro organisms by these processes, although spores of thermophiles may be added from equipment.

During refining of sugar, contamination may come from equipment. Sometimes organisms are added during baggig.

In the manufacture of beet sugar, cleaned beets are sliced and sugar is removed by a diffusion process at 60 - 85°C. Sources of contamination are flume water and diffusion battery waters.

Thermophiles may grow up to 70°C in diffusion battery waters. Granulated sugar now on the market is very low in microbial content for the most part, and ranges from few to several hundred organisms per gram, mostly bacterial spores.

Maple syrup:

Sugar maple sap in the vascular bundles is sterile, but becomes contaminated from outside sources in the tapholes and by the spout, plastic tubing and buckets or other collection vessels. Microorganisms entering sap are mostly psychrotropic, gram negative rods of *Pseudomonas*, *Alcaligenes* and *Flavobacterium*, yeasts and molds.

Paraformaldehyde taphole pellets are inserted into the drilled hole to prevent microbial growth from blocking the flow.

Bacteria counts in sap are usually less than 10,000 per milli liter, but higher numbers can develop as a result of warmer temperatures near the end of the season and poor sanitation.

Honey:

Chief sources of micro organisms in honey are the nectar of flowers and honey bee. Yeasts have been shown to come from the nectar and from the intestinal content of the bee.

Bacteria may come from intestine of bee. Honey rarely contains staphylococci, enteric bacteria. Commonly isolated are acidophilic and glycolytic yeasts. Honey has been found to contain lysozyme (lytic effects on most gram positive bacteria.).

Use of antibiotics such as neomycin and streptomycin is widespread in beekeeping and these antibiotics have been found in the honey obtained from treated larvae and bees. Honey is one of the suspected food vehicles for the source of *Clostridium botulinum* spores in cases of infant botulism. About 10% of the suspected honey samples contained viable spores.

Gluconobacter and *Lactobacillus* are the two main groups of bacteria present during maturation of nectar to honey.

Candy:

Candies from retail markets contain from 0-2 million bacteria per piece. Ex: Coliform bacteria.

Candies receive most of their contamination from their ingredients, air, dust and handling.

Candies and confections can be divided into two categories for microbiological consideration.

Cold processed and Hot processed.

Cold processed candies	Hot processed candies
Molded chocolate and chocolate coatings for creamed centers pasteurization temperature applied during processing.	Hard candy, jellies, caramels, fudges processing temperatures may vary.

Chocolate candies have been incriminated incase of Salmonellosis. Cross contamination occurs in the plant between raw and roasted cocoa beans.

Low moisture content or the dryness of the chocolate apparently protects the salmonellae from heat.

Temperature of 60°C for 10 hrs is commonly applied during processing and blending of milk chocolate.

Preservation

Sugar has normally low a_w (water activity). When moisture has been absorbed by sugar, there is a chance for microbial spoilage. Storage conditions should be such that vermin are kept out and the sugar remains dry. The recommended storage temperature is similar to that for cereals.

Cane or sugar beets may be stored in a controlled atmosphere. Fungal growth is inhibited by 6% CO_2 and 5% N_2 .

During the manufacture of sugar, clarification, evaporation, crystallization, centrifugation and filtration may reduce number of microorganisms.

Chemical preservatives are effective in reducing microbial numbers during sugar refining.

Because of their high sugar concentration and low a_w , most candies are not subject to microbial spoilage, although soft fillings of chocolate of micro organisms.

The bursting of chocolates is prevented by a uniform and fairly heavy chocolate coating and use of a fondant or other filling that will not permit the growth of gas formers.

Sirups and molasses usually have undergone enough heating to destroy most microorganisms but should be stored at cool temperatures to prevent or slow chemical changes and microbial growth. Some molasses may contain enough SO_2 to inhibit microorganisms, but most syrups and molasses contain no added preservatives and prevent microbial growth because of the high osmotic pressure of the sugar solution.

Mold growth on the surface is prevented by a complete fill of the container and is reduced by periodic mixing of the syrup or molasses.

Boiling process during evaporation of maple sap to maple syrup kills the important spoilage organisms. Such syrup, bottled hot and in a completely filled container, usually keeps well.

Honey usually not pasteurized and therefore may be subjected to crystallization and to possible spoilage in time by osmophilic yeasts.

Commercially distributed honey usually pasteurized at 71-77°C for a few minutes. A recommended treatment of heat is 71°C hold for 5 min and cool promptly to 32.2 to 38°C.

Spoilage

Spoilage of sugars or concentrated solutions of sugars is caused by osmophilic yeasts or xerotolerant micro organisms.

Ex: *Saccharomyces*, *Bacillus*, *Leuconostoc*.

Sucrose:

During manufacturing of sugar, juice becomes more concentrated and becomes crystallized. Fewer kinds of organisms can spoil it.

Raw juice:

Raw cane or beet juice is not high in sugar and contains a good supply of accessory foods for microorganisms. Therefore readily deteriorated by the numerous organisms. Gum and slime may be formed.

Ex: Dextrans produced by *Leuconostoc mesenterides* and *Leuconostoc dextranicum*.

Levans produced by *Bacillus* spp. Yeasts or molds are less commonly observed.

Sugar in storage:

Liquid sugar with sugar content as high as 67 to 72 brix will support the growth of yeasts.

Ex: *Saccharomyces*, *Candida*, *Rhodotorula*. Molds enter from the air. Absorption of moisture at the surface may result in growth of micro organisms and hence deterioration of the product. This can be prevented by circulation of filtered sterile air across the top of storage tank or exposure to U.V. lamps.

Molasses and syrups:

Microbial spoilage of molasses is not common because of protective effect of the sugar.

Canned molasses or sirup may be subject to spoilage by osmophilic yeasts that survive the heat process. If air is present on the top surface, molds are infected some kinds of molasses are acid enough to cause hydrogen swells up on long storage.

Maple sap and sirup:

Five chief types of spoilage are recognized

Ropy or stringy sap usually caused by *Enterobacter aerogenes*, *Leuconostoc* spp.

Cloudy, sometimes greenish sap resulting from the growth of *Pseudomonas fluorescens*, along with *Alcaligenes* & *Flavobacterium*. Red sap, colored by pigments of red bacteria

Ex: *Micrococcus roseus* or yeasts or yeast like fungi. Sour sap, caused by variety of kinds of bacteria or yeasts. Moldy sap, spoiled by molds

Maple sirup:

Ropy – *Enterobacter aerogenes*

Yeasty – *Saccharomyces*

Pink – *Micrococcus roseus*

Moldy – *Aspergillus*, *Penicillium*

Sirup may become dark because of alkalinity produced by bacteria growing in the sap and inversion of sucrose.

Honey:

Honey contains more than 25% moisture, sugar content is 70 – 80% (mostly glucose and levulose). Acidity of honey is P^H 3.2 – 4.2. Chief spoiled by osmophilic yeasts.

Ex: *Zygosaccharomyces mellis*, *Z. richteri*, *Z. nussbaumeri*, *Torula (crypto coccus) mellis*.

Molds do not grow well on honey, but certain sps of *Penicillium* and *Mucor* have developed slowly. Most honey yeasts do not grow at laboratory conditions even though high sugar concentration is provided.

Therefore special theories for the initiation of growth of yeasts in honey have been advanced.

1. Honey – being hygroscopic, becomes diluted at the surface, where yeasts begin to multiply and soon become adapted to the high sugar concentrations.
2. Crystallization of glucose hydrate from honey leaves a lowered concentration of sugar in solution.
3. On long standing, yeasts gradually become adapted to the high sugar concentrations.

Critical moisture content for the initiation of yeast growth has been placed at 21%. Fermentation process usually is slow, lasting for months, and the chief products are CO_2 , alcohol, nonvolatile acids which give an off flavour to the honey. Darkening and crystallization usually accompany the fermentation.

Candy:

Most candies are not subject to microbial spoilage because of their comparatively high sugar and low moisture content. Exceptions are chocolates with soft centers of fondant or of inverted sugar which under certain conditions, burst or explode.

Yeasts growing in these candies develop a gas pressure which may disrupt the entire candy or more often will push out some of the sirup or fondant through a weak spot in the chocolate coating. Often this weak spot on the poorly covered bottom of the chocolate, where a cylinder of fondant squeezes out. The defect is prevented by using a filling that will not support growth of the gas formers and by coating the candy with a uniformly thick and strong layer of chocolate.

LECTURE-30

Microbiology of salts and spices, sources of contamination, spoilage and prevention, fatty foods and rancidity.

Fatty foods:

Fats and Oils: The foods made up chiefly of fats and oils, and the fats and oils themselves are subject more often to chemical than to microbial spoilage. Besides the fatty glycerides, natural fats and oils usually contain small amounts of fatty acids, glycerol or other liquid alcohols, sterols, hydrocarbons, proteins and other nitrogenous compounds, phosphatides, and carotenoid pigments.

The chief types of spoilage result from hydrolysis, oxidation, or combinations of the two processes.

Rancidity

The term rancidity sometimes is used for the result of any change in fats or oils that is accompanied by undesirable flavors, regardless of the cause.

Oxidative rancidity: The spoilage due to oxidation, chemical or microbial, is termed oxidative rancidity.

Hydrolytic rancidity: Changes resulting from hydrolysis, by lipases originally present or by those from microorganism termed as hydrolytic rancidity.

Ketonic rancidity: Extensive oxidation, usually following hydrolysis and the release of fatty acids, can result in ketonic rancidity.

Flavor reversion is defined as the appearance of objectionable flavors from less oxidation than is needed to produce rancidity. Oils that contain linolenic acid, fish oils, and vegetable oils, for example, are subject to flavor reversion.

The oxidation of fats and oils may be catalyzed by various metals and rays and by moisture as well as by microorganisms. Hydrolysis by lipases results in fatty acids and glycerol or other alcohols. Fats subjected to either or both of these types of changes may contain fatty oxy and hydroxy acids, glycerol and other alcohols, aldehydes, ketones and lactones; in the presence of lecithin, they may include trimethylamine, with its fishy odor.

Butterfat and meat fats become "tallowy" as the result of oxidation, but butter fat is called rancid when only hydrolysis to fatty acids and glycerol has taken place.

Some of the pigments produced by microorganisms are fat-soluble and therefore can diffuse into fat, producing discolorations ranging through yellow, red, purple, and brown. Best known is the "stamping-ink" discoloration of meat fat caused by yellow-pigmented micrococci

and bacilli. The fat-soluble pigment is an O-R indicator that changes from yellow to green to blue and finally to purple as the fat becomes more oxidized by the peroxides formed by the bacteria. Yellow, pink, and red fat-soluble pigments may be produced by various bacteria, yeasts, and molds.

Molds cause both oxidative and hydrolytic decomposition that result in rancidity. Bacteria causing rancidity of butter cause a similar defect in olive oil. Among the bacteria that can decompose fats are species of *Pseudomonas*, *Micrococcus*, *Bacillus*, *Serratia*, *Achromobacter*, and *Proteus* and among the molds, species of *Geotrichum*, *Penicillium*, *Aspergillus*, *Cladosporium*, and *Monilia*. Some yeasts, especially film yeasts, are lipolytic. Copra and cocoa butter may be spoiled by molds.

Salad Dressings

Salad dressings contain oil which may become oxidized or hydrolyzed and contains to permit microbial growth. Their acidity (about pH 3 to 4) permits most bacteria but favorable for yeasts or molds.

Egg or egg products, pickles, relish, pimientos, sugar, starch, gums, gelatin, spices, and other ingredients may add microorganisms. The three types of spoilage of mayonnaise and similar dressings are (1) separation of the oil or water from the emulsion, (2) oxidation and hydrolysis of the oils by chemical or biological action, and (3) growth of microorganisms to produce gas, off-flavors, or other defects. Darkening often takes place.

The acidity, coupled with the sugar content, about 4.5 percent on the average in the water phase of mayonnaise, is most favorable to yeasts, and cause gassiness.

Species of *Zygosaccharomyces* and *Saccharomyces* spoil mayonnaise, salad dressing, and French dressing. Acid tolerant bacteria will spoil most types of dressing.

Lactobacillus brevis causes gas in a salad dressing. *B. subtilis* and *B. megaterium* causes gas, rancidity, and separation, since they are not acid-tolerant. Yeasts growing with *B. megaterium* could account for the gas. Darkening and separation of Thousand Island dressing with a pH of 4.2 to 4.4 caused by *B. vulgatus* from the pepper and paprika. Molds can grow on salad dressings if air is available and are favored by the addition of starch or pectin to the dressing.

Essential oils: Essential oils or volatile oils are products obtained from the plant kingdom in which the odoriferous and flavoring characteristics are concentrated. These present no spoilage problems but on the contrary may have some preservative effect as ingredients of foods, e.g., mustard, cinnamon, garlic, and onion oils. Most of them do not affect the heat resistance of microorganisms.

Bottled beverages:

Carbonation is inhibitory or even germicidal to some microorganisms, and the acidity resulting from carbonation and the addition of acids, e.g., citric, lactic, phosphoric, tartaric, and malic, inhibits the growth of organisms not tolerant to acidity. Also benzoic acid (75 mg/kg) may be added as a preservative. Nonacid drinks such as root beer are better culture media for spoilage organisms than acid drinks such as the cola drinks, ginger ale, and fruit-flavored drinks. The ingredients of soft drinks not only affect the suitability for microbial growth but also can affect the kinds and numbers of microorganisms. In addition, the bottles and closures are possible sources of contamination.

The water for soft drinks is purified in regard to carbonate and mineral content and is filtered. The filtration process may remove microorganisms. Ultraviolet irradiation sometimes is used to destroy microorganisms in the water. Discoloration of water and a flocculent precipitate may be caused by growth of algae. Treatment with chlorine or chlorine dioxide has been recommended to kill the algae, and filtration, e.g., through diatomaceous earth, to remove the flocculent dead cells.

Yeasts, chiefly *Torulopsis* and *Candida*, are the most likely causes of spoilage of soft drinks, for most such beverages are acid and contain sugar.

Cloudiness results from marked growth of various yeasts or bacteria and ropiness from the development of capsulated bacteria (*Bacillus*). Bacteria may enter from ingredients, bottles, or closures. Occasionally, *Gluconobacter*, *Lactobacillus*, or *Leuconostoc* may be isolated from spoiled soft drinks. *Achromobacter* sps was found responsible for a musty odor and taste in root beer.

Molds require air for growth and they cannot grow on carbonated beverages but may develop at the surface of uncarbonated ones containing air above the liquid.

Spices and other condiments: The dry spices are not normally subject to spoilage, although mold growth during their drying may give them a heavy load of mold spores. Chip dips flavored with vegetables or spices usually have much higher total coliform, and mold counts than those flavored with cheese.

Treatment of the spices with propylene oxide greatly reduces their content of microorganisms. Other treatments to reduce the initial flora would include irradiation, steam, hot ethanol vapors, and acid treatments followed by neutralization.

Prepared mustard can be spoiled by yeasts and by species of *Proteus* and *Bacillus*, usually with a gassy fermentation.

Salt: The three kinds of salt used in foods are (1) solar salt from the evaporation of surface salt water, (2) mined or rock salt, and (3) well salt from salt dissolved from subterranean salt deposits.

Solar salt contains halophiles, such as *Halobacterium salinarum*. About three fourths of the bacteria are *Bacillus* organisms, and the rest are mainly *Micrococcus* and *Sarcina*.

Mined salt has been found to contain about 70 percent *Micrococcus*, 20 percent *Coryneforms*, and 4 percent *Bacillus*.

Nutmeats: Nutmeats in the shell are usually sterile or nearly so. Shelled nuts may be contaminated with bacteria, yeasts, and molds. The test for coliform bacteria is used most often to indicate possible contamination with fecal matter during handling. Roasting and heating in oil or sugar solution reduce the load of microorganisms. Molds may produce mycotoxins on nuts, such as aflatoxin production in peanuts.

Other foods: The number of different food items displayed on a supermarket shelf is proof of the great diversity of commodity items, convenience foods, and prepared foods available.

LECTURE-31

Microbiology of canned foods- Causes of spoilage, appearance of the unopened container, types of biological spoilage of canned foods. Flat sour spoilage, TA spoilage, sulfide spoilage.

Causes of spoilage

Spoilage of heated foods may have a chemical cause or a biological cause or both. The most important kind of chemical spoilage of canned foods is **hydrogen swell**.

Hydrogen Swell results from the pressure of hydrogen gas released by the action of the acid of a food on the iron of the can.

Hydrogen swells are favored by

- (1) Increasing acidities of foods.
- (2) Increasing temperatures of storage.
- (3) Imperfections in the tinning and lacquering of the interior of the can.
- (4) A poor exhaust.
- (5) The presence of soluble sulfur and phosphorus compounds.

Other defects, caused by interaction between the steel base of the can and the contained food include

- (1) Discoloration of the inside of the can.
- (2) Discoloration of the food.
- (3) Production of off-flavors in the food.
- (4) Cloudiness of liquors' or syrups.
- (5) Corrosion or perforation of the metal.
- (6) Loss in nutritive value.

Biological spoilage of canned foods by microorganisms may result from either or both of two causes: (1) survival of organisms after the administration of the heat treatment and (2) leakage of the container after the heat process, permitting the entrance of organisms.

Mild heat treatments permit the successful storage of the foods for limited periods with the help of refrigeration. Examples are processing of meat loaves and pasteurization of milk are examples of such mild heat processes.

Acid foods, such as fruits, are processed at temperatures approaching 100° C, treatments which result in killing all vegetative cells of bacteria, yeasts, and molds and their spores and some bacterial spores. The only survivors ordinarily are spores of bacteria, which cannot grow in a

very acid food. Any survivors of heat treatments by steam under pressure are very heat resistant bacterial spores, usually only one or two kinds.

Microorganisms entering through leaks in containers may be of various kinds and are not necessarily heat-resistant. Leakage and subsequent spoilage of canned food may be a result of mechanical damage of the empty cans so that side and end seams are defective; rough handling of filled cans may also result in damage.

Appearance of the unopened container

1. Flat: Normally the ends of a can of food are termed flat, which means that they are actually slightly concave; and a partial vacuum exists in the container.

If pressure develops inside, the can goes through a series of distortions as a result of increasing pressures and is called successively a flipper, springer, soft swell, or hard swell.

2. Flipper: A flipper has flat ends, one of which will become convex when the side of the can is struck sharply or the temperature of the contents is increased.

3. A springer: A springer has both ends of the can bulged, but one or both ends will stay concave if pushed in; or if a swollen end is pushed in, an opposite flat end will pop out.

The terms "flipper" and "springer" are used by some to designate slight pressures in the can not caused by gas production but by such things as a poor exhaust, overfilling, denting of the can, changes in temperature, etc., but the can may have the same outward characteristics at the start of gas production from either a microbial or a chemical cause or both.

4. A Soft swell: A soft swell has both ends of the can bulged, but the gas pressure is low enough to permit the ends to be dented by manual pressure.

5. A hard swell: A hard swell has such high gas pressure from within that the ends are too hard to dent by hand. Often the high gas pressures distort or buckle the ends or side seam of the cans. The final step is the bursting of the can, usually through the side seam, but sometimes through the seals at the ends.

6. A breather: A breather is a can with a minute leak that permits air to move in or out but does not necessarily allow microorganisms to enter.

Other defects in the general appearance of the can are noted before and after it are opened.

Dents are responsible for a flipper. Other defects are rust perforations, defective side seam or end seals and corrosion.

The glass container of food under gas pressure may have its cover bulged or popped off or may show leakage of food through the broken seal. Of course, it is possible to see evidence of microbial growth through the glass sides, such as gas bubbles, cloudiness, and films of growth.

Grouping of canned foods on the basis of acidity.

The acidity of canned foods is important in determining the heat process necessary for their sterilization and the type of spoilage to be expected if the process is inadequate or leakage takes place. Various groupings of canned foods have been made by the National Food Processors Association, always with a division into the low-acid foods with the pH above 4.5, and a high-acid group, with the pH below 4.5.

Types of Biological spoilage of canned foods

Types of spoilage of canned foods by microorganism usually are divided into those caused by the thermophilic bacteria and those caused by mesophilic microorganisms.

Other methods of classification of kinds of spoilage are based on the kinds of changes produced in the food, e.g., putrefaction, acid production, gas formation, and blackening.

Types of spoilage also may be grouped on the basis of the kinds of foods involved.

The three most important kinds of biological spoilage of commercially canned foods are flat sour spoilage, TA spoilage, and putrefaction.

Hydrogen Swell: A fourth important kind of spoilage, caused by action of food acid on the iron of the can, results in hydrogen swell.

Types of Spoilage by Thermophilic Spore-forming Bacteria

Most spoilage of commercially heat processed canned foods results from under processing and caused by thermophilic bacteria because their spores are more heat resistant than those of most mesophilic bacteria.

The three chief types of spoilage by thermophiles are Flat sour spoilage, TA spoilage, and Sulfide spoilage, or "Sulfide stinker."

Flat Sour Spoilage:

This kind of spoilage derives its name from the fact that the ends of the can of food remain flat during souring, or the development of lactic acid in the food by the flat sour bacteria. Because the can retains a normal outward appearance. This type of spoilage cannot be detected by examination of the unopened can but must be detected by cultural methods.

The spoilage occurs chiefly in low-acid foods, such as peas and corn, and is caused by species of *Bacillus*. Flat sour spoilage of acid foods, e.g., tomatoes or tomato juice, is caused by a special facultatively thermophilic species, *B. coagulans*. The various species of *Bacillus* that are able to form acid without gas in food may be mesophiles, facultative thermophiles, or obligate thermophiles. The spores of the mesophiles are the least heat-resistant and are usually

killed by the heat processing and hence are rarely concerned with flat sour spoilage of low-acid foods.

The spores of the thermophiles are more heat-resistant and may survive the heat process to cause flat sour spoilage. Thermophiles, such as *B. stearothermophilus*, would not cause spoilage unless the food were held hot for a while, as in slow cooling or storage in the tropics, but facultative thermophiles could grow at ordinary temperatures. The immediate source of the flat sour bacteria is usually the plant equipment, e.g., the blanchers, but they may come originally from sugar, starch, or soil.

The ability of *B. coagulans* to grow in tomato juice depends on the number of spores present, the availability of oxygen, and the pH of the juice. The organism, which is homo fermentative under almost anaerobic conditions and hetero fermentative under aerobic conditions, can grow in low concentrations of oxygen.

TA Spoilage (Thermophilic anaerobe)

The bacterium causing this type of spoilage has been nick named T A, which is short for "thermophilic anaerobe not producing hydrogen sulfide," or for the species *Clostridium thermosaccharolyticum*.

This is a thermophilic spore-forming anaerobe that forms acid and gas in foods. The gas, a mixture of carbon dioxide and hydrogen, swells the can if it is held long enough at a high temperature and may eventually cause bursting. The spoiled food usually has a sour or "cheesy" odor. Sources are the same as for flat sour bacteria.

Sulfide, or "Sulfur Stinker," Spoilage

This spoilage, caused by *Desulfotomaculum nigrificans*. This bacterium is found uncommonly in low-acid foods such as peas and corn. The spores of this bacterium have considerably less heat resistance than those of flat sour and T A bacteria; hence their appearance in canned foods is indicative of gross under processing. The organism is an obligate thermophile and therefore also requires poor cooling of the heat-processed foods or hot storage for its development. It is detected by means of the black (FeS) colonies it forms in an iron sulfite agar at 55° C.

Hydrogen sulfide, formed in the canned peas or corn, is evident by odor when the can is opened. In corn, a bluish-gray liquid is evident in which blackened germs and gray kernels of corn float. Peas usually give the H₂S odor but without any marked discoloration. Sources of the spores are similar to those for flat sour and T A bacteria, but manure can also be an original source.

LECTURE-32

Microbiology of Canned foods. Causes of spoilage, appearance of the unopened container. Types of biological spoilage of canned foods - Flat sour spoilage, TA spoilage, sulfide spoilage.

Types of Spoilage by Mesophilic Spore-Forming Bacteria

Most spoilage by mesophilic microorganisms results from under processing is caused by spore-forming bacteria of the genera *Bacillus* and *Clostridium*.

But lightly heated foods, e.g., some acid ones, may permit the survival of, and spoilage by, non-spore-forming bacteria or even yeasts or molds.

Spoilage by Mesophilic *Clostridium* species:

Species of *Clostridium* may be sugar-fermenting, e.g., *C. butyricum* and *C. pasteurianum*, and cause the butyric acid type of fermentation in acid or medium-acid foods, with swelling of the container by the carbon dioxide and hydrogen gas produced. Other species, such as *C. sporogenes*, *C. putrefaciens*, and *C. botulinum*, are proteolytic or putrefactive, decomposing proteins with the production of malodorous compounds such as hydrogen sulfide, mercaptans, ammonia, indole, and skatole.

The putrefactive anaerobes also produce carbon dioxide and hydrogen, causing the can to swell. The spores of some of the putrefactive anaerobes are very heat resistant; therefore, putrefaction joins flat sour and T A spoilage in constituting the chief types of biological spoilage of canned foods resulting from under processing.

Because the spores of the saccharolytic clostridia, sometimes called "butyrics," have a comparatively low heat resistance, spoilage by these anaerobes takes place most commonly in canned foods which have been processed at 100 C or less. Thus canned acid foods such as pineapple, tomatoes, and pears have been found spoiled by *C. pasteurianum*. Such spoilage is more likely when the pH of the food is above 4.5.

Home-canned foods heated to about 100° C may be spoiled by the saccharolytic bacteria with the production of butyric acid, carbon dioxide, and hydrogen.

The putrefactive anaerobes grow best in the low-acid canned foods, such as peas, corn, meats, fish, and poultry.

Spoilage by Mesophilic *Bacillus* species: Spores of various species of *Bacillus* differ considerably in their heat resistance, but in general the spores of the mesophiles are not as resistant as those of the thermophiles. Spores of many of the mesophiles are killed in a short time

at 100° C or less, but a few kinds can survive the heat treatments employed in steam-pressure processing.

Many species of *Bacillus* are aerobic and therefore cannot grow in a well-evacuated container. The food may be too acid for the bacteria, or the medium may be unfavorable otherwise. *B. subtilis*, *B. mesentericus*, and other species growing in low-acid home-canned foods that had been given a heat processing at 100° C.

Commercially canned foods have been spoiled by *Bacillus* species, especially in poorly evacuated cans. Foods so spoiled have been mostly canned seafood, meats, and evaporated milk. The aerobacilli, or gas-forming *Bacillus* species (*B. polymyxa* and *B. macerans*), causes spoilage of canned peas, asparagus, spinach, peaches, and tomatoes, but there is some doubt whether they survived the heat process. Entrance is through a leak in the container. Spores of these bacteria have about the same heat resistance as those of *Clostridium pasteurianum*.

Spoilage by Non-Spore-Forming Bacteria: If viable non-spore-forming bacteria are found in canned foods, a very mild heat treatment was used. Vegetative cells of some kinds of bacteria are fairly heat resistant in that they can withstand pasteurization. Among these thermophilic bacteria are the enterococci, *Streptococcus thermophilus*, some species of *Micrococcus* and *Lactobacillus*, and *Microbacterium*. Acid-forming *Lactobacillus* and *Leuconostoc* species have been found growing in under processed tomato products, pears, and other fruits. The heterofermentative species may release enough CO₂ gas to swell the can.

Micrococci have been reported in meat pastes and *S. faecalis* or *S. faecium* is often present in canned hams that are only partially sterilized and may be responsible for spoilage on storage.

The presence of viable non-spore-forming bacteria in heat processed canned foods indicates leakage of the container by cooling water through tiny orifices e.g., the coliform bacteria, produce gas which swells the cans. It should be noted that spore forming bacteria also can enter the can through a leak, so that the aerobacilli (*B. macerans* and *B. polymyxa*) or the clostridia could be responsible for the gas formation. Non-gas-forming bacteria are found growing in the food in leaky cans, along with the gas former or by themselves. Ex; *Pseudomonas*, *Alcaligenes*, *Micrococcus*, *Flavobacterium*, *Proteus*, and others.

Spoilage by Yeasts:

Since yeasts and their spores are killed readily by most pasteurization treatments, their presence in canned foods is the result of either gross under processing or leakage. Canned

fruits, jams, jellies, fruit juices, syrups, and sweetened condensed milk have been spoiled by fermentative yeasts, with swelling of the cans by the CO₂ produced.

Film yeasts may grow on the surface of jellied pickled pork, repacked pickles or olives, and similar products, but their presence indicates recontamination or lack of heat processing, plus poor evacuation.

Spoilage by Molds:

Molds are the most common cause of the spoilage of home-canned foods, which they enter through a leak in the seal of the container. Jams, jellies, marmalades, and fruit butters will permit mold growth when sugar concentrations are as high as 70 percent and in the acidity usually present in these products.

It has been claimed that adjustment of the soluble extract of jam to 70 to 72 percent sugar in the presence of a normal 0.8 to 1.0 percent acid will practically remove the risk of mold spoilage. Strains of *Aspergillus*, *Penicillium*, and *Citromyces*, found growing in jellies and candied fruits, are able to grow in sugar concentrations up to 67.5 percent. Some molds are fairly resistant to heat, especially those forming the tightly packed masses of mycelium called sclerotia. *Byssoschlamys fulva*, a pectin-fermenting mold, has ascospores that have resisted the heat processing of bottled and canned fruits and have caused spoilage.

Spoilage of Canned Foods of Different Acidities:

1. The low-acid foods with a pH above 5.3 are especially subject to flat sour spoilage and putrefaction.
2. Foods with a pH between 5.3 and 4.5 are likely to undergo TA spoilage.
3. High-acid foods with a pH between 4.5 and 3.7 usually are spoiled by a special flat sour bacterium or by a saccharolytic anaerobe.
4. High-acid foods with a pH below 3.7 ordinarily do not undergo spoilage by microorganisms, but in cans may become hydrogen swells.

Spoilage of Canned Meats and Fish:

In general, canned meats and fish exhibit two chief types of spoilage:

- (1) By *Bacillus* species, resulting in softening and souring.
- (2) By *Clostridium* species (e.g., *C. sporogenes*), producing putrid swells.

Bacilli may produce acid and gas and swell the cans less commonly.

Mostly spore formers that have survived the heat process enter through leaks.

Canned cured meats which are given a heat process insufficient for sterilization, such as ham or luncheon meats, may be subject to production of carbon dioxide, nitrogen oxides, or

nitrogen gas by species of *Bacillus* (e.g., *B. licheniformis*, *B. coagulans*, *B. cereus*, or *B. subtilis*) from the nitrate, sugar, and meat, or they may be subject to putrefaction with gas produced by *Clostridium* species. Such spoilage ordinarily is prevented by adequate refrigeration.

Gas also produced by hetero fermentative lactic acid bacteria (e.g., *Leuconostocs*), but only after inadequate processing. Spoilage without gas production but with souring and changes in color and texture may be caused by species of *Bacillus* and by homo fermentative lactic acid bacteria (e.g., *Streptococcus faecium* or *faecalis*).

Unusual Types of Spoilage of Canned Foods:

Sulfide spoilage has been reported only for peas and sweet corn.

Black beets are caused by the mesophilic *Bacillus betanigrificans* in the presence of a high content of soluble iron. This is distinct from the darkening resulting from a deficiency of boron in the soil.

Bacillus species can cause bitterness, acidity, and curdling in canned milk, cream, and evaporated milk.

The only important alkaline canned vegetable, hominy (pH 6.8 to 7.8), undergoes flat sour spoilage which is characterized by a sweetish taste.

Canned poultry is more often spoiled by putrefactive than by saccharolytic clostridia, chiefly because of the lower heat resistance of spores of the latter.

Canned sweetened condensed milk may become gassy because of growth of yeasts or coliform bacteria, may become thickened by a *Micrococcus* species, or may exhibit buttons, which consist of small masses of mold mycelium and coagulated milk usually on the surface of the milk. The size of the buttons is limited by the quantity of free oxygen in the head space of the can.

Sometimes, as the result of auto sterilization, no viable organisms can be found in cans of food that have undergone obviously biological spoilage. All vegetative cells have died, including those of spore formers which did not sporulate.

